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ADRENAL CORTEX AND WATER METABOLISM[1,2]

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THE ROLE OF THE ADRENAL CORTEX in electrolyte and water metabolism was the first of the important adrenal cortical functions clearly recognized when studies of cortical physiology began to take precise form in the early 1930's. Emphasis has been placed, however, upon the actions of cortical hormones on electrolyte metabolism, to which their actions on body water were generally considered as a secondary osmotic consequence. In particular, the fact that the cortical hormones cause sodium retention, and hence water retention, led to the idea that their major action on water metabolism was one of water conservation.

Within recent years, on the other hand, it has become clear that a definite function of cortical hormones is to stimulate water diuresis. In a water-loaded animal this action can be a dramatic life-saving one. Under proper circumstances the water-eliminating effect of cortical hormones is of comparable magnitude to that of the water-conserving action of their physiological counterpart, the posterior pituitary antidiuretic hormone.

The dilemma which presents itself is, therefore, that the cortical hormones may cause either water retention or water loss. This is not an unusual situation because many substances exert different effects depending upon the setting of the physiological stage on which they operate. Evidence now at hand is sufficient to permit an intelligible if not necessarily complete interpretation of these different aspects of adrenal cortical function.

I. ADRENAL CORTEX AND ELECTROLYTE METABOLISM

No attempt will be made here to review comprehensively the extensive literature on the effect of the adrenal hormones on electrolyte metabolism, since that has recently been done (241, 95, 122). A summary will be given, however, of data basic to subsequent considerations.

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[2] Abbreviations used in this review: ADH = posterior pituitary antidiuretic hormone; DCA = desoxycorticosterone acetate; ACTH = adrenocorticotrophic hormone of the anterior pituitary.

While considerable earlier information existed (71), the reports of Loeb *et al.* (134) and Harrop *et al.* (93, 91) in 1933 gave convincing demonstration of the fact that adrenal insufficiency was characterized by renal loss of sodium salts. The work of many investigators provided the details showing that a renal wastage of sodium, chloride and bicarbonate characterize adrenal insufficiency in most animals and in man although species differences (223, 215, 21) may exist. This loss was sufficient to explain some, but not all, aspects of the adrenal insufficiency syndrome and in addition provided a rational basis for the demonstration of the therapeutic benefits of salt feeding in adrenal insufficiency (231, 133, 135, 186) and increased salt appetite after adrenalectomy (178, 180). Of the known cortical steroids DCA repairs the sodium deficiency most effectively and when given in overdosage will cause excess retention of this ion (92, 241). An action of exceedingly small quantities of DCA can be detected on the excretion of radioactive sodium (48). It has also been found that the sodium and chloride content of sweat is high in Addisonian patients and in normal individuals is reduced by either DCA or ACTH (39).

The possibility is subsequently discussed that some of these apparent actions of the cortical hormones on NaCl metabolism are directly due to an imbalance between adrenal cortical and posterior pituitary activity.

When it became evident that alterations in sodium metabolism would not explain all the phenomena seen in adrenal insufficiency (237), attention was focused on potassium which may increase both in the serum and tissues after adrenal removal (241, 122). The adrenalectomized animal is highly sensitive to the toxic effects of high serum levels of potassium, as it is to other deviations from a normal internal or external environment, and in certain instances potassium accumulation presumably contributes to death in adrenal deficiency. Again DCA most effectively stimulates the excretion of potassium and given in excess may reduce plasma potassium to a dangerous level in the dog (125), man (255) and probably in the rat (81). Such reduction is accompanied by sodium retention and by the replacement of cellular potassium by sodium (55). These findings together explain well the fact that the best maintenance of adrenalectomized animals, aside from hormone replacement therapy, is to provide a high sodium–low potassium diet (2).

It is generally accepted that the kidney tubule is the primary site of action of the corticoids as far as their effects on sodium and potassium excretion are concerned. They facilitate the reabsorption of sodium and inhibit the reabsorption of potassium (89, 241, 183); the opposite occurs in adrenal insufficiency. The curious observation has been made (183), however, that when high plasma sodium levels are maintained in adrenalectomized dogs by infusion, the reabsorption of sodium is increased above normal—just the opposite of what occurs when plasma sodium is within normal or sub-normal ranges.

Certain of the gonadal steroids simulate the action of DCA in causing sodium retention (252), but judging by earlier experiments all cortical steroids do not. In acute experiments some corticoids, e.g., corticosterone and dehydrocorticosterone (250, 35), are weak sodium retainers and some,17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone (compound E), enhance sodium excretion (251, 225a). Chronic experiments revealed that the latter effect is a transitory one (114, 225a).

Recent work shows that generalization on the specific action of any cortical steroid is very difficult. Forsham *et al.* (60), for instance, have found that compound E, which under some conditions induced sodium excretion, would cause mild sodium retention in Addisonian patients; this effect was antagonized to a certain extent by the addition of DCA! On the other hand, ACTH returned toward normal the altered sodium and potassium plasma levels induced by DCA in normal rats (279). Further confusion is provided by the demonstration that chronic use of DCA may cause in certain conditions a negative sodium and potassium balance with elevated water exchange (152, 283).

It is obvious that the over-all effects of the secretions of a normal adrenal on electrolyte metabolism may be different and hardly predictable from the actions of individual steroids. The action of a given steroid is dependent upon dosage, duration of treatment, other steroids present and additional less well defined physiological variables. As will be noted later, however, all adrenal preparations that have been studied stimulate water diuresis in normal and water-loaded animals.

II. EFFECT OF ADRENAL CORTICAL HORMONES ON WATER EXCRETION

Rowntree and Snell (185), from observations of human patients, were the first to call attention to the fact that water was not excreted normally when given to an Addisonian subject. Subsequent isolated observations were made by several workers and more recently extensive studies on the subject have been made both in laboratories and clinics.

Response to Fluid Administration in Adrenalectomized Animals and Addisonian Patients. Just as is true of ADH, the actions of cortical hormones on water excretion are best seen if observed in an animal or patient in a hydrated state. Regulatory mechanisms for eliminating excess water are then brought into play and if deficient are readily detected.

Animal experimentation on the subject was initiated by Silvette and Britton (214) who showed that adrenalectomized cats excreted either water, urea solution, or salt solution, given intraperitoneally, at a markedly delayed rate. The inference was drawn that the effects were of extra-renal origin and due to some avidity of the tissues for water.

Rigler (181) showed that the administration of water, glucose solution, hyper- or hypotonic NaCl solution to adrenalectomized mice failed to elicit the expected diuresis. Swingle *et al.* (238) obtained similar results with water administration in adrenalectomized dogs, as did Gaunt, Remington and Schweizer (77) in both adrenalectomized and hypophysectomized rats. All of these workers noted, in addition, a marked susceptibility to water intoxication after adrenalectomy, a susceptibility later shown to be in part due to extra-renal factors (Sec. VI).

In subsequent work the essential fact that animals or men with either a marked or latent adrenal insufficiency cannot excrete water at a normal rate has been abundantly confirmed (140, 142, 124, 216, 278, 196, 123, 211, 182, 100, 108, 213, 230, 82, 183, 136, 242) and details of the phenomenon elaborated.

The most thorough studies have been made in the rat. At 18 hours after adrenalectomy, this species will excrete a dose of water used conventionally in diuretic

experiments (approximately 4–5 cc/100 gm.) at a normal average rate, but the variability between individuals will be considerably greater than in normal animals (69). If the water load is increased by giving 2 to 5 such doses of water at hourly intervals, a marked deficiency in diuresis is evident and symptoms of water intoxication, not seen in normal animals, intervene (68). If tested 3 to 7 days after adrenalectomy an inability to excrete even the mild single hydrating dose of water is readily apparent (69). This might not be surprising if adrenal insufficiency with its attendant circulatory and metabolic dysfunctions were developing spontaneously during the post-adrenalectomy interval, but with rare exceptions (123) the phenomenon is almost equally marked in those animals maintained from the time of operation in what would usually be considered a 'normal' state by salt, DCA or adrenal cortical extract (68; see also Sec. VII).

Details have been worked out less completely in species other than the rat, but such information as is available indicates that the same pattern holds for the mouse (181, 111), dog (238, 242), opossum (216), hamster (49) and man (172, 182). In the human species there are only a few (128) recorded exceptions to the rule that Addisonian patients fail to excrete ingested water or injected glucose or saline solution normally.

It has also been found that influences which normally augment the diuretic response to ingested water are either ineffective or of reduced effectiveness in the absence of cortical hormones. This is true in experimental hyperthyroidism (70), in the augmentation of diuresis that follows adaptation to excess water (130) and after the administration of epinephrine (73) or KCl (140). A deficiency of cortical hormones may contribute to the appearance of experimentally-induced edema (107), and injection of these hormones into rats will relieve the deficiencies in diuresis and susceptibility to water intoxication seen in hypophysectomy (119) and in both pantothenic acid and riboflavin deficiency (74).

Effects of Cortical Hormones on the Diuretic Response to Water in Normal Animals. When diuresis is induced in normal rats by a single dose of water given by stomach tube, its rate of elimination is enhanced by the administration of either DCA (205, 158) or adrenal cortical extract (158). The same is true when cortical extract is used in the opossum (217). Similar results have been seen following chronic treatment with DCA in the dog (148), but this was in the case of animals developing a diabetes insipidus-like state. Such effects are much more clearly seen if water is given in multiple doses adequate to produce water intoxication. Under these conditions, diuresis is markedly stimulated by cortical hormones and it is exceedingly difficult to induce lethal water intoxication (66, 78). In intact but not in the adrenalectomized animals this protection afforded against water intoxication is apparently due largely to effects of cortical hormones on the kidney; after adrenalectomy extra-renal factors can also be detected (Sec. VI). The stimulation of water diuresis and the protection against water intoxication provides one of the most easily demonstrated overdosage actions of the adrenal cortical hormones, and in severe water intoxication also provides one of the few definite demonstrations of a life-maintaining action of these hormones against lethal stress in normal animals.

In the frog, a form in which the response to adrenalectomy and adrenal cortical

hormones is aberrant and ill-understood in other respects (241), cortical hormones cause a retention, rather than elimination, of excess injected water (109).

Daily Water Exchange in Adrenalectomized Animals and Effects of Replacement Therapy. The facts outlined above show deficiencies in the excretion of water forcibly administered to adrenalectomized animals. This type of observation has to be correlated with the fact that in the spontaneous daily water exchange, slightly increased urine volume is a characteristic but not invariable symptom of the early and mild stages of adrenal insufficiency in the untreated dog (134, 93, 240). In the rat urine volume may vary from polyuric to oliguric levels after adrenalectomy, depending on the severity of symptoms and other variables not clearly defined (9, 187, 191, 76, 41). Water intake changes less than urine output, resulting in some cases in a reduced water intake/urine volume ratio. This early diuresis following adrenalectomy is not seen in the cat (270) and as adrenal insufficiency becomes severe, oliguria generally supervenes in all species. The post-adrenalectomy water diuresis is associated with and presumably due to the well-known sodium diuresis that follows loss of adrenal function (144a; Sec. I).

It is clear that the kidney can excrete water in amounts somewhat above normal without cortical hormones, but it cannot excrete fluid at anything approaching *normal maximal* rates, when there is need to do so, as after forcing fluid. It has, in other words, lost its adaptability (172) not only for handling water in excess but in dehydration (124) as well.

When dogs are revived from crises of adrenal insufficiency, there occurs promptly a diuresis of considerable magnitude relative to fluid intake (240, 90). This is presumably associated with a return of blood volume to normal and elimination of accumulated metabolites. When adrenalectomized rats are maintained on NaCl therapy, there is an increased water exchange (76, 99, 41) of similar magnitude to that which follows the same treatment in normal animals. Replacement therapy with cortical extract may result in an essentially normal or slightly elevated water exchange (76), the details of the response probably being dependent on the effective hormone dosage used. DCA therapy produces an increased water exchange after adrenalectomy (41). When DCA and salt therapy are combined a high water intake and output results which is much greater than with either treatment alone (41, 99).

Effects of Cortical Hormones on Daily Water Exchange of Normal Animals. In proper experimental circumstances, DCA can produce a polydipsia and polyuria in normal animals. This effect, however, varies somewhat in different species and is dependent on the salt intake in some way only vaguely understood. In rats on a normal or low salt intake, DCA has been reported to increase water intake and output by some workers (205, 276, 25, 99, 41, 209, 6) and not to affect it by others (171, 174). All workers who have looked for it, however, have seen tremendous water exchanges in rats given DCA plus a high NaCl intake (41, 171, 99, 25, 174, 276, 209, 81). The original work on the subject was done by Kuhlman *et al.* (125) in the dog where moderate doses of DCA induce a diabetes insipidus-like condition which is also dependent to a considerable extent on the salt intake (171, 148). Confirmation of the work on dogs (272, 147) and demonstration of a similar diabetes insipidus-like

condition following DCA therapy in a human case of myasthenia gravis has been recorded (147). Interpretation is tending toward the idea that the polydipsia rather than the polyuria is primary (171, 148, 174, 160, 3). If so, the DCA-induced condition differs fundamentally from true diabetes insipidus. As discussed later, however, direct effects of DCA on the renal tubular reabsorption of water are probably also involved. In cats, on the other hand, DCA will not affect the water exchange either in the normal or diabetes insipidus animal (272).

The fact that DCA will reduce the intake of NaCl solution after adrenalectomy in self-selection experiments (180) and increase it in normal animals (174) seems paradoxical at the moment.

There is only a single but nevertheless convincing instance known to the authors in which a diabetes insipidus-like condition has been seen following the use of cortical preparations other than DCA. Ingle (113) gave young fifty-gram rats up to 20 cc. per day of adrenal cortical extract in drinking water for 28 days. Growth rate was reduced but no glycosuria appeared. A polydipsia developed exceeding 100 cc. of water intake per day.

In intact dogs given a *constant* fluid and food intake, cortical extract produced variable effects on water excretion while reducing the excretion of sodium and increasing that of potassium (92).

Patients with cardiac disease and associated water retention show a marked diuresis after the administration of large doses of lipo-adrenal extract (132).

Effects of Cortical Hormones on Water Excretion in Dehydrated Animals. Results cited above show that the cortical hormones increase diuresis in animals in normal water balance and in those either mildly or heavily hydrated. Adrenal cortical extract will also increase water excretion in rats mildly dehydrated by deprivation of food and water for 20 hours (158). DCA under similar conditions failed to influence urine volume (158) but it may do so in the dehydrated state after it has been chronically administered in the dog (148) or the rat (276).

III. MECHANISM OF ACTION OF CORTICAL HORMONES ON WATER METABOLISM

Renal Factors. The well known alterations in renal function (241, 222, 282, 95, 63, 10, 168, 7, 143, 117) which occur in adrenal insufficiency make it logical to suspect that the kidney itself may be the major site of cortical hormone activity in regulating diuresis. Until recently, however, only fragmentary evidence unsuitable for comprehensive conclusions was available on the point.

Some cases of renal pathology associated with adrenal insufficiency have been described (7, 138, 97, 220). Other workers, however, have reported the absence of such pathological changes or have not observed them frequently enough to explain the consistent alterations in renal function seen in adrenalectomized animals and Addisonian patients (80, 245, 83, 8, 234). In addition, cortical hormones have effects on renal tubules in tissue culture (28).

Decreased clearances of urea, creatinine, and phenolsulphonphthalein have been a long-recognized characteristic of adrenal insufficiency (228, 227, 143, 88, 10, 63, 89). While some studies on adrenalectomized animals (214) and Addisonian patients (245) indicate normal function by ordinary clinical tests for renal disease, more precise observations indicate definite disturbances. Modern methods have shown a

decreased renal plasma flow (245, 264, 62, 266) and glomerular filtration in both Addison's disease and experimental adrenal insufficiency (89, 141, 152, 245, 80, 192, 264, 266, 15); such factors could contribute to a depressed water diuresis. Waterhouse and Keutmann (264) find essentially normal filtration fractions in treated Addisonians despite reduced filtration rates; from this they postulate a reduction in the effective vascular bed. The rate of glomerular filtration will tend to become adapted to varying water loads after adrenalectomy in rats, increasing as larger amounts of water are ingested (15). At any water load, however, the rate of urine flow is depressed more than expected from the decrease in the filtration rate (15). Furthermore, the deficiencies of filtration in adrenal insuffiiency can be wholly or partially corrected by either salt (136, 266, 62, 88, 89, 16) or adrenal hormone treatment (141, 152, 245, 264, 89, 16, 192); yet, at least in the absence of heroic therapy, the deficiencies in water excretion remain (136, 68, 142, 16; cf. Secs. VII and XII). The conclusion follows, therefore, that there is an increased tubular reabsorption of water in adrenal insufficiency. The same conclusion was reached by Gersh and Grollman (80) on the basis of cytochemical observations of the kidneys of adrenalectomized rats and Silvette and Britton (217) from inferences on urea excretion. Nelson and Berman (155), studying histological preparations of kidneys from adrenalectomized rats that had been forced into water intoxication in Gaunt's experiments, reported that "the absence of specific glomerular damage and dilation of Henle's loops may indicate that filtration can occur even in those cases where there is practically no urine output."

The accumulation of an antidiuretic substance in the blood of adrenalectomized animals or an increased sensitivity to such substances (13), as subsequently discussed, makes an increased tubular reabsorption of water not only understandable but expected.

The next question is whether an overdosage of cortical steroids will have the opposite action, i.e., whether it will inhibit water reabsorption in the tubules of normal animals. Despite some dissent (193), most direct evidence on the point gives an affirmative answer. Winter and Ingram (272) found that DCA will decrease tubular reabsorption as judged by creatinine clearance both in normal and diabetes insipidus dogs. Similar results were obtained in rats using both DCA and adrenal cortical extract (16) and in a human case given DCA (283). In some circumstances, it increased filtration as well (272, 38, 193). The effect on tubular reabsorption in normal animals is apparently not the result of a reduction in the blood levels of antidiuretic substances (6), and there is other evidence that the factors operative in depressing diuresis in adrenal insufficiency are not complete counterparts of those that enhance diuresis after administration of cortical steroids in normal animals (12).

Although changes in renal circulation and glomerular filtration may be contributory factors, it seems safe to conclude that the effects of cortical hormones on tubular reabsorption constitute their major action on water excretion. This function cannot be correlated with the renal hypertropic action of steriods (205, 50) since the substance, testosterone, which most effectively stimulates renal hypertrophy does not induce polyuria (205). Neither will testosterone repair the renal deficiencies in Addisonian patients.

Alterations in the metabolism of kidney slices from adrenalectomized animals

have been reported (44, 253, 254). Russell and Wilhélmi (188, 189), for instance, found that kidney slices show both a subnormal oxygen uptake and deamination of amino acids after adrenalectomy and that these functions may be restored to normal by the administration of cortical extract or DCA. The relation of these facts to the excretion of water is obscure. Since the deficiencies in water excretion, unlike those on oxygen uptake and deamination, are very difficult to correct with replacement therapy a functional relation between them is questionable.

It is possible that the hormones which regulate diuresis could act by altering patterns of renal blood flow according to the scheme of Truetta and co-workers (257) but evidence on the point is lacking.

Relation between Water and Electrolyte Metabolism as Affected by Cortical Hormones. The knowledge that some cortical hormones cause sodium retention, and thus indirectly water retention, has to be correlated in some way with the fact that these same hormones are diuretics and essential for the process of normal water excretion. The possible relation, therefore, of the diuretic action of cortical hormones to electrolyte metabolism becomes one of great interest.

Many workers have agreed that, *a*) there is a failure of the diuretic response to force-fed water in adrenal insufficiency regardless of whether the salt loads of the body are high or low, *b*) that salt solutions are excreted only little if any better than pure water in the absence of cortical hormones, and *c*) that the action of cortical hormones on water excretion in adrenalectomized and normal animals is not necessarily associated with any apparent causal alteration in salt excretion (181, 172, 140, 142, 214, 66, 68, 82, 242). The same things are true in hypophysectomized animals (119). Furthermore, DCA, the adrenal steroid most effective in causing salt retention, is still diuretic in its action under most circumstances (Sec. II). From this it might be concluded that the cortical hormones exert some direct effect on water excretion, independent of their actions on electrolyte metabolism. The fact that DCA and other adrenal hormones inhibit the renal tubular reabsorption of water suggests the necessary mechanism. Such an idea would embrace current information concerning the opossum—a form which shows unorthodox effects of cortical hormones on electrolyte excretion but which demostrates, nevertheless, the expected diuretic response to these hormones (217).

While the cortical hormone-induced diuresis of acute experiments in adrenalectomized animals seems to be unaffected by salt loads, the diabetes insipidus-like syndrome, induced by chronic administration of DCA in intact animals, although said to be "primarily a disturbance in water metabolism" (148), is at least to some degree dependent on salt intake. In the rat, the syndrome is demonstrable only slightly (205, 25) or not at all (171, 174) in the absence of some undetermined amount of dietary salt, but is readily seen when salt is given concomitantly with DCA (171, 25, 174). In the dog, the effect may not be entirely dependent on excess dietary salt, but it is so in its full-blown manifestations (148, 171). Other experiments demonstrate that in normal, adrenalectomized or hypophysectomized animals, the diuretic effects of DCA, chronically administered, were either dependent on or greatly increased by extra NaCl intake (41, 99). It is important, however, that all these examples of induced polyuria were associated with polydipsia and possibly secondary

to it. It has been suggested (148, 171, 174, 160, 3) that the action of DCA in this respect is primarily extra-renal in origin and results from a vicious cycle in which the steriod induces salt retention and thereby thirst, a process exaggerated by additional dietary salt. According to this concept polydipsia would be the immediate cause of the polyuria. Such ideas apparently would be consistent with the work of Rice and Richter (174).

To accept the hypothesis that the polyuria induced by chronic administration of DCA is primarily due to polydipsia does not, however, preclude the possibility that an additional contributory factor is a DCA-inhibited tubular reabsorption of water, although the latter factor may perhaps be overshadowed by a more massive polyuria induced by thirst. It is suggested, therefore, that the various cortical hormones enhance urine volume in two ways; *a*) *they augment thirst due to sodium retention.* In this effect DCA is the most active adrenal steroid because of its sodium-retaining action. Its diuretic effects consequently are best seen in chronic experiments, allowing time for a salt load to be built up, and are vastly enhanced by added dietary salt. In animals deprived of water, the effect may not be seen at all (158) unless extensive prior treatment with DCA (148, 276) has built up a water and salt load. *b*) In addition, the cortical hormones *inhibit tubular reabsorption of water.* This effect is essentially independent of salt load and is most easily seen under conditions of acute water overdosage, but can still be detected after water deprivation and dehydration (158).

If this hypothesis be granted, it would appear to permit correlation of most existing data on the subject—data which has hitherto seemed enigmatic (69). It also would permit intelligible recognition of the fact that DCA by a primary and dominant sodium-retaining action could, on the other hand, cause a reduced water excretion (148, 151, 193); such might be expected transiently in the absence of excess water when salt is retained before a polydipsia appears. There are other instances in which the action of DCA is reversed when the physiological conditions are changed, e.g., it depresses salt excretion in Addison's disease and enhances it in Cushing's Syndrome (225).

IV. RELATION BETWEEN ANTIDIURETIC HORMONE OF NEUROHYPOPHYSIS AND ADRENAL CORTICAL HORMONES

The theory that normal water balance is the consequence of a physiological antagonism between the diuretic action of cortical hormones and the antidiuretic action of posterior pituitary hormone was first stated (218, 217) and extensively elaborated (42, 216, 41, 20, 18) by Britton and collaborators. A great deal of additional work indicating the antagonistic action of cortical and posterior lobe hormones on water excretion (148, 131, 115, 4, 6, 272, 274, 268, 183), and as shown below on NaCl excretion, can be marshalled but the concept of a simple check-and-balance relationship should be approached with caution (210) until further evidence is available. It has not been shown, for instance, that secretions of the two glands are released temporally and quantitatively in a way suggesting an integrated physiological relationship. And while some of their actions are antagonistic, others are not, e.g., both DCA and Pitressin enhance potassium excretion at normal or low levels of potassium intake (194).

A possible new approach to the problem has been provided by the recent discovery that an antidiuretic substance, which behaves like posterior pituitary ADH, can be readily detected and measured in the blood serum of normal rats (13, 11) and other species (hamster and rabbit, 14; man, 132). The properties of this substance (13, 116, 11) suggest its identity with ADH: *a*) its antidiuretic action is effected by enhancing the tubular reabsorption of water although it transiently increases glomerular filtration; *b*) it is chloruretic; *c*) it is increased in dehydration and decreased by hydration; *d*) it is not detectable in the serum of hypophysectomized rats; *e*) it is, like Pitressin, quickly inactivated by neutralized thioglycollic acid. Its specificity is implied by the fact that isotonic gelatin solutions have no similar antidiuretic effects, while the antidiuretic actions of crude irritating protein solutions can be clearly distinguished from those of serum by the fact, among others, that they are not chloruretic. It will be interesting to explore the relation of this finding to that of the labile oxytocic substance found in human blood (43).

This antidiuretic substance was found to be increased after adrenalectomy (13), a result in harmony with the earlier report (144) that an antidiuretic substance appeared in the urine of cats after adrenalectomy. This presumably accounts in part for the increased tubular reabsorption of water and failure of water diuresis after adrenalectomy but on the basis of present evidence cannot do so completely. For instance, when large doses of water are force-fed to rats within 18 hours after adrenalectomy, deficiencies in diuresis are already evident but there is not yet appreciable accumulation of the antidiuretic substance. Secondly, when adrenalectomized rats are treated with saline or small doses of DCA the levels of antidiuretic substance are restored to normal but deficiencies in diuresis persist (6). There is another factor, however, which may be of greater importance than the elevated blood levels of antidiuretic materials in adrenal insufficiency. Properly devised tests have shown that within 18 hours after adrenalectomy there is a clearly enhanced sensitivity to the injection of antidiuretic substances (Pitressin) (13). An increased sensitivity to such materials would result in a depressed diuresis after water ingestion whether or not the amounts were above normal.

Lloyd's studies (132) attempting to correlate the blood levels of antidiuretic materials with corticoid excretion in a variety of clinical conditions, although incomplete as yet, offer a particularly promising approach to the problem. He finds low levels of unidentified antidiuretic substances in the serum of patients with diabetes insipidus, high levels in Addison's disease, and in general high urine volumes are associated with high corticoid excretion and vice-versa.

The early experiments of Martin and co-workers (144) led them to the conclusion—entirely consistent with their observations—that the presence of a urinary antidiuretic after adrenalectomy reflected a purposeful adaptation of neurohypophyseal activity to prevent dehydration. When it is considered, however, that forced fluids cannot be excreted at normal rates after adrenalectomy, it seems more probable that the organism is dealing with an unregulated aberration of normal function.

The recent report of Skahen and Green (209) that the chronic administration of DCA or salt leads to hypertension, polyuria and the excretion of an antidiuretic substance in the urine cannot at the present time be correlated with observations

noted above. It seems paradoxical that both an overdosage and deficiency of cortical steriod hormone should induce accumulation of antidiuretic substances in body fluids. Could it be, however, that the chronic administration of either DCA or salt would lead to a compensatory secretion of ADH to facilitate (teleologically speaking) the excretion of salt? There is abundant evidence of an antagonistic action of cortical and posterior pituitary hormones on NaCl secretion (131, 41, 42, 4, 152, 210, 194), just as there is on water excretion. The cortical substances, particularly DCA, cause NaCl retention and Pitressin enhances its excretion. Under conditions of rapid water diuresis a chloride-retaining action of cortical hormones is difficult to demonstrate, but the chloruretic action of Pitressin, considered either in terms of concentration or total amounts of chloride in the urine, has been seen by many above-mentioned workers. Questions both of fact and interpretation have been raised, however, in regard to such results (85, 161, 86). The demonstration by Winter, Ingram and Gross (274, 268) that the depletion of plasma sodium which follows adrenalectomy in cats is dependent upon a functional posterior pituitary is of great interest and significance in this connection. The implication of these various results is that the posterior pituitary is fully as important as cortical hormones in regulating NaCl metabolism and that normal salt and water excretion may result from a balanced interaction of the two glands. It is questionable whether this theory will explain the increased tubular reabsorption of sodium that occurs in adrenalectomized dogs when high plasma levels of sodium are maintained (183).

No satisfactory information is available as to the cause of the accumulation of antidiuretic substances in the blood of adrenalectomized animals. It could be due to an increased posterior pituitary activity, although Gersh and Grollman (80) failed to find cytological evidence of such an increase, or to an arrested rate of inactivation of ADH. The liver is known to be involved in the inactivation of ADH (103, 52) and liver functions in other respects are altered by adrenalectomy (185, 248) and cortical hormones (221). While the possibility of causal relations is suggested, evidence is lacking. Current theory has it that ADH is released by the stimulus of a rising plasma osmotic pressure (29, 27, 259). It is highly unlikely that such factors are operative after adrenalectomy, particularly after the ingestion of excess water where most important osmotically active substances are reduced in concentration. Even if it be granted that osmotic stimuli are not present to activate the release of ADH, other stimuli might conceivably do so. It is known, for instance, that neurogenic as well as osmotic stimuli may excite the neurohypophysis (17, 156, 98).

The hypophysectomized animal is an experimental form of interest from the standpoint of possible antagonism between the adrenal cortex and posterior pituitary. Here one gland is extirpated and the other largely inactivated from lack of its trophic hormone. Unfortunately the situation is complicated by the fact that non-adrenotrophic anterior pituitary hormones are also lacking. Whatever may be true of the daily water exchange and the phenomenon of diabetes insipidus after total hypophysectomy, as discussed below, one fact is clear: the diuretic response to forced water is sluggish after hypophysectomy as it is after adrenalectomy (263, 32, 119, 165, 157); the rate of extra-renal water loss—itself low in the absence of the anterior pituitary—exceeds the rate of renal excretion (119); and the deficiency responds

markedly to cortical hormone therapy in the rat (119, 157) if not the dog (165). The renal abnormalities of hypophysectomized dogs, in other respects, are not repaired by cortical hormones (267). In addition, there is a marked susceptibility of hypophysectomized rats (77) and frogs (197) to water intoxication and an abnormal response to the fluid shifts induced by the injection of intraperitoneal glucose (118) which resemble to some degree conditions seen in adrenal insufficiency. This led to the hypothesis that the failure of water diuresis was due to identical causes in both adrenalectomy and hypophysectomy (119). Recent work requires that this hypothesis be revised. As noted earlier, the adrenalectomized animal has a higher than normal amount of antidiuretic substance in the blood; the hypophysectomized animal, on the other hand, has none (11). The two specimens are therefore fundamentally different as far as the factors acting on water metabolism are concerned. These facts permit understanding of the hitherto anomalous observation (119, 157) that the diuretic response to water is restored by cortical hormones more easily in hypophysectomized than in adrenalectomized animals. In the latter, any injected material has to counteract a large amount of circulating antidiuretic substance; in the former it can act unantagonized. In the absence of known agents inhibiting water excretion it is surprising that the hypophysectomized animal cannot excrete large volumes of water readily. One can only state in explanation the general rule that rapid water excretion is apparently never possible in the absence of cortical hormones. These facts do not fit comfortably into the simple hypothesis that the rate of water excretion is determined only by the relative amounts of cortical and posterior pituitary hormone present in the blood. If both are absent rapid diuresis is still impossible.

Wesson, Anslow and Smith (265a) have offered an hypothesis relating the action of ADH to sodium and water excretion and to the regulation of extracellular volume. They did not attempt to fit the action of cortical hormones into their scheme.

V. RELATION OF ADRENAL CORTEX, ANTERIOR PITUITARY AND POSTERIOR PITUITARY IN DIABETES INSIPIDUS

Another approach to the relation of cortical and pituitary hormones in water metabolism is that provided by the extensive studies on experimental diabetes insipidus. It is now generally accepted that full-fledged diabetes insipidus results when a neurohypophyseal mechanism is damaged or inactivated and the anterior pituitary functional (262, 79, 179, 58). When the whole pituitary is removed a transitory diabetes insipidus develops which within a few days may subside to negligible proportions. Disturbances in water metabolism persist, however, as indicated by a low ratio of water intake to urine output (199), by a persisting inability to excrete a concentrated urine (162, 198), and by a diminished diuretic response to water ingestion in acute experiments (Sec. IV).

Of particular interest for this discussion is the nature of the essential rôle of the anterior pituitary in the maintenance of diabetes insipidus. No specific anterior lobe diuretic hormone has been found, and most workers feel that the effect is mediated by the indirect action of one or more of the known anterior lobe hormones (177).

The theory that ACTH, acting through the adrenal cortex, is the diuretic agent of the anterior pituitary was first stated by Britton *et al.* (218, 217). Other workers (198, 199, 272, 105) have not accepted the idea in toto. The implication of much of the evidence cited in foregoing sections of this review is, however, that the adrenal cortex must be involved to some degree in the maintenance of diabetes insipidus. The important points of argument can be briefly stated as follows:

1) The cortical hormones are natural diuretic agents and are essential to the full action of other natural diuretics such as thyroxin (198, 70) and epinephrine (73). Since anterior pituitary removal largely inactivates the adrenal cortices, the absence of full-fledged diabetes insipidus after hypophysectomy is to be expected. *2*) The removal of the adrenals diminishes the diabetes insipidus established by appropriate hypothalamic lesions (115) and overdosage with DCA accentuates the water exchange characteristic of diabetes insipidus (99). *3*) Cortical hormones, particularly DCA (198, 152, 205, 104, 41), will partially restore a state of diabetes insipidus in hypophysectomized animals but this action is not as quick or complete as would be expected if this were the sole influence involved (198). *4*) In intact dogs, chronic administration of DCA can induce a diabetes insipidus-like syndrome (Sec. II).

The evidence previously cited of adrenal cortical-posterior pituitary antagonism leads logically to the concept that total hypophysectomy, by removing or functionally inactivating these two glands, attenuates diabetes insipidus in a manner analagous to that in which hypophysectomy attenuates diabetes mellitus. To admit the importance of these adrenal cortical factors does not, however, permit dismissal of impressive evidence that other factors are involved in diabetes insipidus. The thyroid, which is more completely inactivated than the adrenals by hypophysectomy, is an important agent in water metabolism and its rôle in diabetes insipidus has been amply demonstrated (58, 105, 121, 23, 33).

The growth hormone of the pituitary affects water metabolism as an essential feature of its growth-promoting action and in addition has profound effects on renal function (267a); it thus is probably involved in this problem. Since the animal without a functional neurohypophysis cannot concentrate urine, any pituitary hormone which stimulates appetite or metabolism in such a way as to require the excretion of additional metabolites must cause added water excretion. The enhancement of diabetes insipidus when additional salts (232, 273, 233, 275) or urea (273, 271) have to be excreted has been frequently observed.

There are, therefore, several anterior pituitary hormones which tend to produce polyuria. When they are unchecked by ADH, diabetes insipidus is probably the resulting sum-total of their activities.

VI. EXTRA-RENAL ASPECTS OF ADRENAL CORTICAL REGULATION OF WATER METABOLISM

The previous sections have been concerned largely with factors affecting the excretion of water and electrolytes. Such factors lead obviously to changes in internal fluid distribution. Some of the internal changes which follow adrenalectomy are secondary to altered renal functions and some are not. The distinction is oftentimes difficult to make. Here again the problem involved is that of the intrinsic or self-

contained antagonistic properties of steriods such as DCA; they cause sodium retention and water retention, while at the same time affecting kidney tubules in such a way as to stimulate water excretion.

Earlier reviews (241, 95, 122) contain more complete accounts of the older work on this subject than will be attempted here.

Internal Fluid and Electrolyte Distribution; Water Intoxication; Intestinal Absorption. An appearance of dehydration is a commonly described symptom of adrenal insufficiency (224). A careful study of the total body water in the adrenalectomized rat, however, revealed no significant reduction in the percentage of water determined on a fat-free basis (87). There is a reduction in blood volume (93, 281, 239, 96) and of extracellular fluids in general (93, 91). While it has been suggested that the loss of blood volume is probably due to loss of fluid through the kidneys (93, 91), this explanation is open to question as it has been estimated that the water and NaCl (59) loss from the extracellular fluid is greater than can be accounted for on the basis of renal excretion (235, 241, 90). A shift of water from extra- to intracellular sites would be expected if this were true and has been found (90, 46). The latter is reflected by an increased water content of all tissues studied: muscle (261, 214, 212, 45, 150), skin (94), liver (214, 94), red blood cells (149, 102).

Overdosage phenomena correlate well with the above findings. Many observers have reported an increased blood volume following DCA administration in intact animals (3, 236, 170, 37, 36), while others have observed that various adrenal steroids prevent the decline in blood volume after adrenalectomy (236, 19, 129, 127). In the case of DCA administration this is associated with sodium retention and potassium loss, and after prolonged treatment with the substitution of sodium for intracellular potassium (55). It has been reported (65) recently that DCA given to normal dogs caused an increase in the extracellular space with no change in total body water. This means that there was a decrease in the intracellular water. In contrast, dogs treated with cortical extract showed no significant change in the extracellular volume but the total body water, and hence intracellular water, increased markedly.

The effects of DCA on both electrolyte and water retention are apparently antagonized to some extent by other cortical secretions (Sec. I), a fact which serves as the basis for the observation that edema is easily produced by DCA therapy in Addisonian (56) but not in normal patients (224, 249). Similarly, an excess of natural adrenal secretions presumably accounts for the fact that in Cushing's Syndrome, DCA enhances the rate of excretion of intravenous NaCl solution whereas it facilitates its retention and consequent edema formation in Addisonians (225).

A definite example of the extra-renal origin of some phenomena of this type is seen in water intoxication. Adrenalectomized animals show a sensitivity to excess water not wholly due to deficient water excretion (242, 100, 68). That point was unmistakably made by the demonstration that after nephrectomy DCA greatly increased the resistance to high water loads (12). An analagous resistance to water intoxication, apparently not due entirely to increased diuresis, was seen in animals adapted to the receipt of water by stomach tube (130, 247). Such adaptive phenomena were not manifest after adrenalectomy (130). The protective action of cortical hormones against excess water in normal animals (66), however, is due largely to renal factors (12). The nature of the extra-renal actions of the cortical hormones in water

intoxication is not clear but much basic information has been reported (181, 238, 242, 68, 100, 211, 12) and summarized (69). Briefly, marked differences have been reported between normal and adrenalectomized animals given excess water in the maintenance of body temperature, hematocrit values, plasma electrolyte levels, intestinal absorption, etc. These differences are only partially explicable by renal activity and in general suggest an abnormal internal distribution of ingested fluid after adrenalectomy. Convincing exposition of the nature of that difference has not been made and current opinions, even as to the site of retained fluid, are not in complete agreement (68, 211, 100, 69).

Water intoxication is generally thought to be due to cellular hydration resulting from a reduction by dilution or otherwise in extracellular electrolyte. It can be prevented by NaCl administration (184, 84, 67). It might be expected, therefore, that the cortical hormones would prevent water intoxication by maintaining high plasma electrolyte levels due to their usual salt-retaining actions. It is a curious fact that they do not exert their effect, whatever it may be, by this means (238, 66, 12) either in normal or adrenalectomized animals. On the other hand, thyroxin, which also prevents water intoxication, does probably act in part by causing salt retention (70).

Another unexpected fact is that lethal water intoxication is not associated with evidence of circulatory collapse in adrenalectomized animals (238, 68) as is the case with most other stresses of similar severity. Circulatory abnormality of a more subtle sort, however, is a possible cause of the recent finding that there is a sharp fall in renal glomerular filtration when adrenal deficient animals are forced into water intoxication (14).

One sharply defined extra-renal factor is a deficiency in the intestinal absorption of sodium salts and water after adrenalectomy (34, 68, 47, 229) and hypophysectomy (119). This could be a major cause of the sluggish water diuresis but actually is probably of minor importance; as indicated in earlier sections, fluid given by parenteral routes is excreted as poorly as that which has to go through the gut.

Permeability. Viale and Bruno (261) observed a decrease in the plasma volume of adrenalectomized dogs and suggested increased vascular permeability as a possible explanation. Other early work implied changes in capillary permeability in adrenal insufficiency (239). The failure of serum transfusions to protect adrenalectomized animals against circulatory collapse, and the appearance of edema following such transfusions (236), indicated increased capillary permeability. Cope *et al.* (40) observed an increased protein content of lymph collected from the cervical trunk of dogs after adrenalectomy—a result probably indicating changes in capillary permeability. Hyman and Chambers (110) found that various cortical steroids helped prevent edema in the perfused legs of frogs. It has been reported (269) and questioned (167) that muscle tissue of adrenalectomized rats and of frogs (5) imbibes water faster in hypotonic solutions and loses water faster in hypertonic solutions than do muscles taken from normal animals. These findings may have been the result of equilibrium phenomena caused by differences in initial electrolyte content of the tissues studied rather than manifestations of permeability changes per se. A large volume of experimental evidence (145, 61, 57, 241, 95) is concerned with changes in permeability to dye substances after adrenalectomy.

While much has been said or implied concerning the effects of adrenal cortical

hormones on permeability, knowledge of the extent and nature of such phenomena is far from satisfactory. Most convincing evidence concerns capillary permeability to proteins. Whether these effects are all due to permeability changes per se or to changes in circulatory dynamics is open to question (30). The problem of possible changes in cellular permeability to electrolyte and other ions remains unanswered. While it is clear that cortical hormones bring about changes in ionic distribution between cells and extracellular fluids (Sec. I), these seem to be the direct result of alterations in the electrolyte content of extracellular fluids, a consequence of renal activity, rather than an expression of hormone action on cellular permeability.

VII. COMPARATIVE ACTIONS OF DIFFERENT STERIODS ON WATER METABOLISM; REPLACEMENT THERAPY

Although DCA has long been recognized as the cortical steroid that acts most effectively on the kidney to inhibit salt excretion and increase potassium excretion and has been known as the prototype of the adrenal 'salt and water' hormones, it is not the most effective in stimulating diuresis in acute tests or in preventing water intoxication. One mg. of 17-hydroxy-11-dehydrocorticosterone (compound E) has a potency about equal to 3 mg. of DCA in maintaining diuresis and preventing toxic symptoms in the adrenalectomized rat given large doses of water (54). Under similar conditions whole adrenal cortical extract is clearly superior to DCA when used in doses standardized to equivalence in life and growth maintenance tests (54). Cortical extract is much more effective than DCA in maintaining the diuretic response to ingested water in hypophysectomized rats (119, 157). It has also been found that while both cortical extract and DCA will protect normal rats from water intoxication the former is apparently more effective (66). In mildly dehydrated animals, the diuretic action of cortical extract has been seen under conditions where DCA is ineffective (158). While further data on the point are needed, it appears now, on the other hand, that DCA is more active than other cortical substances in inducing high rates of water excretion after chronic administration in animals allowed free access to water. Under these conditions a polydipsia is induced that leads to a diabetes insipidus-like syndrome, likely due in part to salt retention (Secs. II, III).

Despite such quantitative differences, all cortical substances which have been tried (DCA, 17-hydroxy-11-dehydrocorticosterone, the amorphous fraction and whole adrenal extract) can stimulate diuresis (54). Progesterone is the only substance exhibiting marked corticoid activity reported to be without effect on diuresis when given acutely in water tolerance tests (75). Re-study of this point is appropriate since it has been shown (206) that, after chronic administration, progesterone enhances water exchange in both normal and hypophysectomized rats and increases the rate of elimination of water given by stomach tube. In the hypophysectomized animals it also increased the resistance to water intoxication (206).

The estrogens and androgens share with many other steroids the property of reducing the excretion of sodium and of causing water retention (252). Like DCA and progesterone they also cause reduction of serum and muscle potassium (146). The estrogens differ from progesterone in that they are toxic to adrenalectomized animals (195, 26, 72). Both estrogen and testosterone differ from progesterone in

that they do not provoke diuresis under any conditions yet observed (75, 205). There is as yet no basis on which all of these facts can be correlated satisfactorily, but probably all steroid hormones affect salt and water metabolism in some way.

One of the most curious aspects of the whole problem is the difficulty of repairing the deficiencies in water diuresis of adrenalectomized animals, and Addisonian patients. Replacement therapies are relatively effective if used in the early stages after adrenalectomy (54, 100), but not if 3 or more days are allowed to elapse after operation. Even when animals are treated with salt, DCA or cortical extract beginning immediately after adrenal removal it has generally not been possible to restore completely the diuretic response to water (100, 142, 68, 82). The same problem has appeared in treated Addisonian patients (128, 69, 280). Large doses of cortical substances are of some effect (68, 100) and it has recently been found (14) that if adrenalectomized rats are treated with 5 mg. DCA per day for 5 days a normal excretion of ingested water is obtained. The dog apparently responds better than other forms (242, 123). The difficulty of replacement is likely related to that of other persisting abnormalities of renal function in Addisonian patients on otherwise adequate therapy (245, 264). Despite the difficulty of restoring diuresis, cortical hormones are, however, effective in preventing symptoms of water intoxication in adrenalectomized animals by their extra-renal action (Sec. VI).

VIII. RELATION OF ADRENAL CORTEX AND MEDULLA IN WATER METABOLISM

In most experiments on water metabolism done with adrenalectomized animals, the adrenal medulla as well as the cortices have been removed and this fact in itself makes inquiry into the possible effects of medullectomy imperative. The recent findings of Verney and associates (260) that epinephrine inhibits the emotional release of ADH adds interest to the problem. (Is it possible that the Verney phenomenon could be due to the fact that epinephrine injections, as is well known, induce the release of ACTH and that the resulting secretion of adrenal cortical hormones antagonizes ADH?)

Some time ago, it was shown that rats in which one adrenal was removed and the other demedullated excreted excess water less rapidly than normal animals. While this deficiency could have been due to the lack of medullary tissue, the fact that it was fully corrected by the use of adrenal cortical extract led to the interpretation that it was rather the result of subnormal functional capacity of the remnant of cortical tissue (53). Obviously more conclusive experiments were needed. The adrenal medulla is activated by water overdosage as indicated by a hyperglycemia which fails to occur after medullectomy (68).

Stein and Wertheimer (230) made the first systematic study of the problem with the finding that medullectomized rats had a deficient diuretic response to water and increased susceptibility to water intoxication. These deficiencies could be repaired, not only in medullectomized but also in totally adrenalectomized rats, by epinephrine injections. Epinephrine increased chloride excretion, particularly in medullectomized rats. Somewhat variable alterations appeared in the daily water exchange of animals from which the adrenal medullae were removed.

While Stein and Wertheimer's interpretations of these findings were conservative,

it is obvious that many of the results previously attributed to cortical deficiency might in fact have been due to medullary deficiency.

Reexamination of this question was made in two laboratories. It was found in rats given variable doses of water, that epinephrine would enhance diuresis, in some cases to a remarkable extent, in normal (73) medullectomized (73) and adrenalectomized (73, 100) rats, and in all groups would enhance chloride excretion (73). The medullary hormone was less effective in preventing water intoxication than expected, perhaps because of its deleterious stimulation of salt excretion.

That the results were of great physiological significance was questioned (73) largely on the basis of the fact that it was not confirmed that demedullated animals responded to forced water in any way different from normal ones, i.e. no deficiency phenomena were observed. Furthermore, these effects of epinephrine injections were seen only when dosage was high enough to produce obvious overdosage symptoms. Amounts of epinephrine which produced no overt overdosage symptoms failed to affect diuresis (73). Scattered reports have been obtained which indicate that epinephrine is of no avail in restoring the diuretic response to water in human cases of adrenal insufficiency (69).

While it is the authors' opinion that the adrenal medulla is not involved in any important respect in the alteration of water metabolism associated with adrenalectomy, there are admittedly unsolved aspects of the problem of great interest. For instance, epinephrine administration may induce either diuresis or antidiuresis and the pharmacology of these different actions is vague (175, 176, 256, 260, 190, 31, 277). It is one of the few non-steroid agents which exerts a clearly diuretic influence in adrenalectomized animals and this action is apparently a specific one; at least other related drugs will not duplicate epinephrine action (230).

IX. RELATION OF ADRENAL CORTEX AND THYROID IN WATER METABOLISM

It has long been recognized that the thyroid influences water metabolism (163, 164), and specifically that thyroxin stimulates water diuresis (164, 70). Since the adrenal cortical hormones stimulate diuresis and thyroxin increases adrenal cortical activity (246, 137) it is obviously possible that the thyroid action might be mediated through the adrenals. Evidence on that point is not conclusive but as shown below it is clear that while the thyroid influence is not exerted entirely through the adrenals, it cannot manifest itself in the absence of cortical hormones.

When animals treated with thyroxin are given water by stomach tube they excrete it rapidly and if a high water dosage is used, a marked resistence to water intoxication is seen (70). This is partly due to the fact that the hyperthyroid animal can excrete large volumes of water of very low chloride, and presumably sodium content (169, 70), an effect which in water intoxication would have the same protective action as that of the administration of salt. This salt conservation is not due to adrenal influence since under similar conditions adrenal cortical hormones do not decrease the chloride excretion (66). Nevertheless, the accelerated diuresis is largely abolished by adrenalectomy and is therefore dependent in some way on adrenal function (70). The effects of hyperthyroidism in man are apparently the opposite of those in animals and lead to decreased rather than increased water tolerance (128). Such

results could be readily explained if hyperthyroidism led to adrenal exhaustion in one species but not another; the question would be an interesting one to explore. It has, in fact, been reported that a reduction in adrenal size may characterize human hyperthyroidism in contrast to the usual hypertrophy in animals (126).

Thyroid hormones affect various renal functions (104, 51) but the possible relation of these actions to the cortical hormones and water metabolism is not clear.

Curiously enough the response to either small or large doses of water is completely normal in hypothyroid rats whether that hypothyroid condition is induced by surgery (101) or thiouracil (70).

X. RELATION OF ADRENAL CORTEX TO ALTERATIONS IN WATER METABOLISM SEEN IN ADAPTATIONAL SYNDROME

When animals are subjected to stimuli which invoke intensive adaptive responses, such as exposure to cold, traumatic shock, drugs, toxins etc., the initial or 'shock phase' of the syndrome is characterized by the retention of administered fluid (107, 120, 108, 6, 22). This condition lasts for approximately 24 hours and is followed by a reverse effect which appears during or just after the 'counter-shock phase' (6, 22, 24). Although careful analyses have not been made of the initial phase characterized by an antidiuretic response to water it is probably of complicated origin; the general shock-like symptoms, hypotension, hemoconcentration, possible increased activity of the neurohypophysis, and possible transient adrenal cortical deficiency due to the rapid utilization of the hormone all may be contributing factors. The latter factor is mentioned because at least in some instances diuresis can be restored (108, 107, 69), and symptoms improved (207) by cortical hormones. During the initial antidiuretic reaction elicited by a stress-producing stimulus the application of a second stress fails to cause further water retention but rather provokes an increased urine output (120, 108, 107).

Within two days after a damaging stimulus has been applied, during what Selye (201, 202) would term the 'stage of resistance,' there is a definite acceleration of the diuretic response to administered water (6, 22, 24). Considering that adrenal enlargement (204, 112) and increased corticoid excretion (258, 265, 166) follow exposure to a wide variety of stress-producing agents (204, 112), it is possible that the diuretic reaction is due to hyperactivity of the adrenal cortex. Further evidence as to the rôle of the adrenal cortex in provoking the diuretic reaction is found in the observation that the response is abolished by adrenalectomy (208, 200, 204) or hypophysectomy (200, 204), and is very closely duplicated in normal animals by adrenal cortical extract (6, 16).

XI. ACTION OF CORTICAL HORMONES IN EPILEPTIC AND OTHER CONVULSIONS RELATED TO TISSUE HYDRATION

McQuarrie and Peeler (154) introduced and others have used (64, 106) a test for idiopathic epilepsy based on a susceptibility to seizures following a low salt diet and acute hydration. In electro-shock and drug-induced convulsions, increase in cellular hydration lowered the threshold to psychomotor seizures and changes in extracellular fluid volume, when unaccompanied by changes in electrolyte concentra-

tion, had no effect on such thresholds (244). Later work suggested that the extracellular sodium and chloride concentrations were more important than several other factors studied (243). The implication that the susceptibility to convulsions is related to such phenomena suggests a possible therapeutic value of a hormone such as DCA which causes sodium retention and helps maintain extracellular fluid volume.

It has been reported (153) that DCA will antagonize the seizure-provoking effects of Pitressin hydration in the epileptic subject and possibly, although there is lack of agreement on the subject (1), decrease the tendency for spontaneous occurrence of grand mal seizures. It likewise increased the threshold to electroshock seizures in the rat (279, 226), an effect which was surprisingly antagonized by adrenal cortical extract or ACTH (279). In the cat, it afforded protection against convulsions induced by cocaine but not electricity (1), and in the rat prevented both convulsions and pulmonary edema after metrazol treatment (203). Such actions resembled those of DCA in preventing water intoxication symptoms, among which are violent convulsions. This relation is further indicated by the fact that the alterations of the electroencephalogram are similar in water intoxication and epilepsy and certain phases of the former at least can be relieved by DCA (78). While the beneficial effects of DCA in epilepsy have been attributed to NaCl retention (153), it is not clear that the same explanation will suffice in water intoxication (12).

Although no comprehensive statements concerning the relationship between these various convulsive states and adrenal cortical function in electrolyte and water metabolism can be made as yet, the possibilities suggested are intriguing. This is particularly true in view of other accumulating evidence, not obviously germane to this review, that cortical hormones have important functions in regulating the electrical activity of the brain (60).

XII. WATER DIURESIS TESTS OF ADRENAL CORTICAL FUNCTION

Many of the deficiencies which occur in adrenal insufficiency have been used as bases for the bioassay of adrenal cortical preparations.

Despite its mechanical simplicity no thorough study has been made of the possible usefulness of the deficient diuretic response to water and its repair, although one report (159) attests its value. It is apparent from published work that dose-response relationships of some sort could be obtained (54). It would be one of the most generalized tests for corticoid activity since most corticoids stimulate diuresis (Sec. VII). One obvious difficulty would lie in the poor responsiveness (Sec. VII) of animals that have been operated on 3 or more days previously. This might be eliminated, however, by basing tests on the enhanced diuresis which follows cortical hormone administration in intact animals.

Wide use has been made, however, of the deficiency in diuresis as a means of testing adrenal function. More or less apart from the concomitant animal work, the clinical findings of Rowntree and Snell (185) and Maranon *et al.* (139) preceded the widely used Robinson-Power-Kepler (182) 'water test' or 'Kepler test' for Addison's disease. The first part of this test simply applies the fact that in the absence of adequate amounts of cortical hormone, ingested water is not excreted at a normal rate. If it is excreted at a normal rate, Addison's disease can be ruled out in most instances. If excretion is deficient, factors other than Addison's disease must, of course, be

eliminated. More recent studies concern the physiological basis, application and reliability of the test (172, 128, 69, 280, 219). As is true in animals (Sec. VII), a normal response to the test is not restored by ordinary replacement therapy. It is apparent from animal work that the accuracy of the procedure could be considerably enhanced if doses of water larger than those recommended (2% of the body weight) could be forced on the human subject (69). Unfortunately, most subjects cannot tolerate larger doses.

XIII. CONCLUSIONS

The following is offered as the authors' tentative interpretations of the data reviewed. Like most hypotheses in a rapidly developing field, they will certainly have to be revised as further information accumulates.

The hormones of the adrenal cortex have an important rôle in water metabolism, which is mediated, broadly speaking, in three different ways. *First*, there is a direct action on renal mechanisms, essentially independent of electrolyte metabolism, the effect of which is to stimulate the rate and extent of water excretion. *Secondly*, most cortical hormones cause sodium retention by the kidney, the osmotic consequence of which is a retention of water. Whether increased or decreased diuresis results from the interaction of these two factors depends upon the physiological conditions prevailing at any particular time. In the excretion of sodium chloride and water, the effects of cortical hormones are the opposite of those of the posterior lobe of the pituitary gland. More data is needed to establish the hypothesis that the adrenals and neurohypophysis operate as a coordinated antagonistic system but to a certain extent they probably do so. Shannon states ". . . both hormones are involved in the control of sodium balance. However, the action of the two does not appear to be integrated in the ordinary sense of the word" (210). The problem invites further attention. *Thirdly*, there are vaguely-defined extrarenal means by which the cortical hormones affect internal fluid distribution.

Diuretic Action of Cortical Hormones. The normal diuretic response to water, when water is given by any means or with any solute, depends upon the action of cortical hormones. Diuretic agents or influences thus far studied can act to their full capacity only in the presence of cortical hormones. Deficiencies in diuresis are apparent when gross symptoms of adrenal insufficiency are absent. Adrenalectomized animals can, of course, excrete water, but not at anything approaching a maximal normal rate. The water excretion of intact animals, even when dehydrated, is increased by the administration of cortical hormones. It is curiously difficult to obtain an effective replacement therapy and restore normal rates of water excretion either in animal or human subjects with adrenal insufficiency.

Actions of the cortical hormones on water excretion can be demonstrated which are not in any apparent essential way related to electrolyte stores or electrolyte excretion. All cortical substances studied have a diuretic influence, despite their variable effects on salt excretion, but compound E is a more effective diuretic than DCA. This means that substances most effective in inducing diuresis are least effective in causing salt retention, and this fact again points to the divorcement of the two actions.

Two actions of the cortical hormones are responsible for their direct effects on

water diuresis. *First*, they are necessary for the maintenance of a normal renal glomerular filtration rate. This is associated with the maintenance of a normal renal plasma flow. The factor of glomerular filtration, however, is of minor importance, since filtration can be restored to normal in situations where deficiencies in water diuresis persist. *Secondly*, the cortical hormones inhibit the renal tubular reabsorption of water and this is the *sine qua non* of their diuretic action. Tubular reabsorption exceeds normal in adrenal insufficiency and is less than normal when intact animals are overdosed with cortical hormone.

Relation of Adrenal Cortex and Posterior Pituitary. The probable cause of the increased tubular reabsorption of water in adrenal insufficiency is an accumulation of, or an increased responsiveness to, circulating antidiuretic substances. Increased amounts of antidiuretic material have been seen in the urine of adrenalectomized cats and in the blood serum of adrenalectomized rats and Addisonian patients. Considerable evidence points to their pituitary origin. In any case, when the diuretic cortical hormones are absent, there is an increased sensitivity to posterior pituitary ADH. A subnormal output of ADH, theoretically at least, could bring about, therefore, antidiuresis during adrenal insufficiency because it would be acting in the absence of a normal antagonist.

An important distinction should be drawn, however, between the analagous antidiuretic rôle of ADH and the diuretic rôle of cortical hormones: ADH is secreted at a variable rate and is sensitively responsive to varying states of hydration. At present there is no evidence to show that mild fluctuations in water load alter the rate of cortical hormone secretion, although such possibilities have not been excluded. Within fairly wide limits, an increased output of ADH may antagonize the effects of cortical hormones and maintain water balance at a more or less normal urine volume. ADH administration, however, is much less effective in controlling a diabetes insipidus-like condition induced by DCA than it is in ordinary diabetes insipidus. This is a counterpart of the increased effectiveness of ADH after adrenalectomy. Presumably, then, when the cortical hormone level is high enough, water balance can only be attained by increased water intake. According to this hypothesis, the cortical hormones would be essential for diuresis, but the finer regulation of water exchange would depend on factors controling thirst mechanisms and ADH secretion. When both the corticoids and ADH are reduced in body fluids, as in hypophysectomy, rapid diuresis is impossible although readily restored by cortical extract. This demonstrates again the essential rôle of the cortical hormones for a high rate of water excretion, whatever may be the nature of their interacting function with ADH.

In diabetes insipidus, the action of cortical hormones, uninhibited by ADH, is almost certainly contributory to the observed polyuria. Anterior pituitary factors, other than the ACTH-adrenal cortical system, however, seem to have important influences as well. The essential rôle of the anterior pituitary in maintaining a state of diabetes insipidus when the neurohypophysis is removed or inactivated is best explained as being due to multiple influences the sum total of which achieves polyuria.

Water-Retaining Action of Cortical Hormones. Most cortical hormones, and particularly DCA, cause retention of sodium salts. This leads to an osmotic retention of water, increased extracellular fluid volume and possibly frank edema. In the early

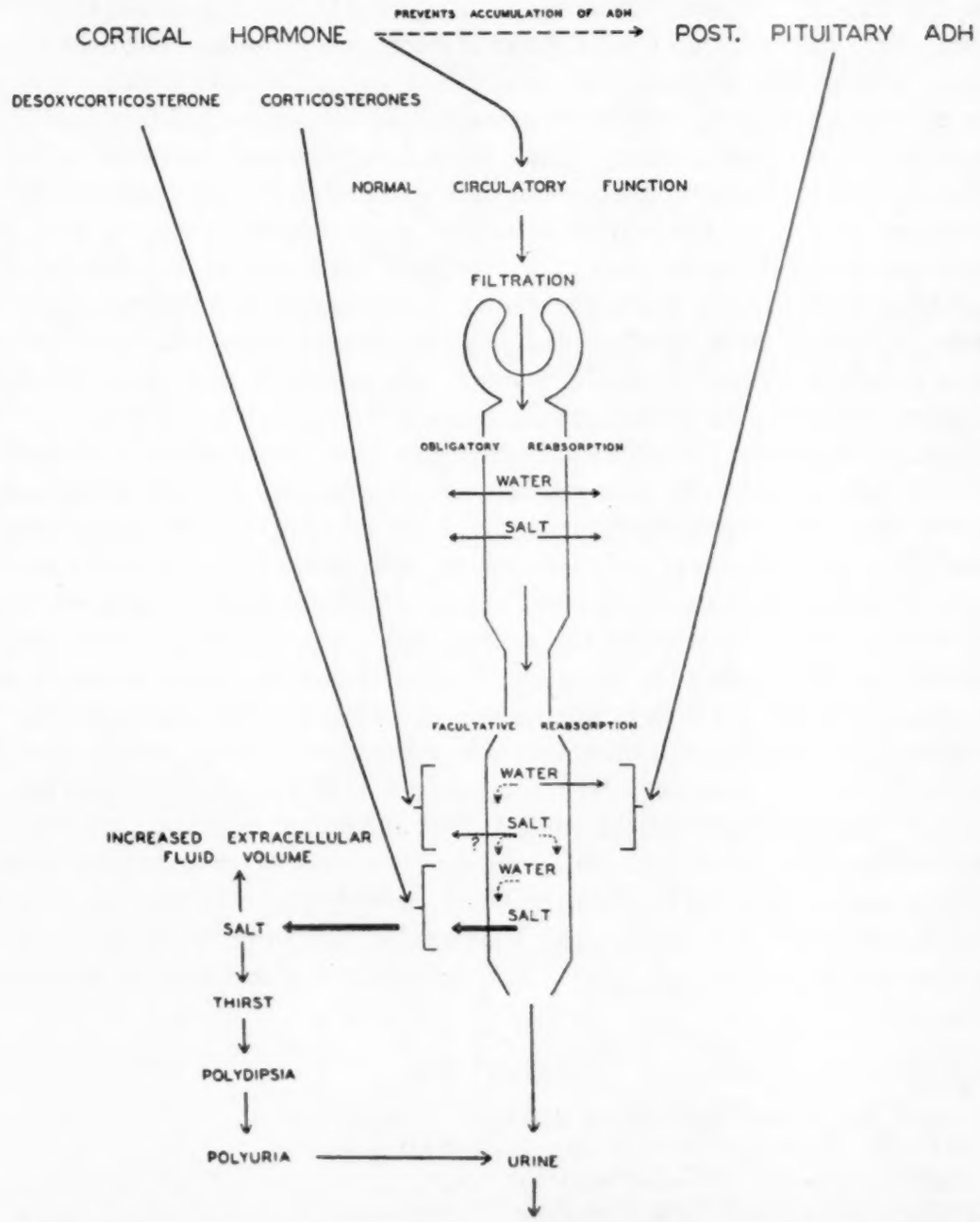

Fig. 1. INDICATING POSSIBLE PATTERN of interaction of adrenal cortical and posterior pituitary hormones in salt and water metabolism. According to this scheme ADH stimulates the tubular reabsorption of water and inhibits (broken arrow) the reabsorption of sodium and chloride. The cortical hormones *a*) prevent the accumulation of antidiuretic material, presumably ADH, in body fluids by mechanisms as yet unknown; *b*) maintain renal plasma flow and glomerular filtration; *c*) inhibit the tubular reabsorption of water; *d*) in some instances and conditions inhibit and in some stimulate the reabsorption of NaCl; *e*) when sodium is retained it causes expansion of the extracellular fluid volume, thirst, and eventually, acting together with other factors, augments the water exchange.

stages after cortical hormone administration, if excess water is not forced on the subject, this water-retaining effect may override the diuretic effect of inhibited tubular reabsorption and result in reduced urine volume. If treatment is continued, however, the retention of salt is associated with the development of thirst which

leads to a diabetes insipidus-like state—a state probably due to a combination of increased thirst and inhibited renal tubular reabsorption of water. When the experimental circumstances are such that these factors can all operate simultaneously, a water retention, i.e., an expanded extracellular fluid volume, and an increased water diuresis can occur simultaneously. Thus, there is no essential inconsistency in the statement that cortical hormones promote both water retention and water loss (fig. 1).

Extrarenal Factors. The extrarenal actions of cortical hormones on such phenomena are too poorly understood to be fitted into this scheme as yet. Their absence is associated with evidence of an altered internal distribution of fluid and a relatively unimportant reduction in the absorption of water from the intestine. Whatever may be their nature, these extrarenal effects are of such magnitude as to make the difference between survival or death when water is given nephrectomized animals. They presumably account for the marked sensitivity to water intoxication in adrenal deficient animals, a sensitivity that results in the appearance of toxic symptoms at relatively light water loads. Further study of the relation of these phenomena to permeability, the behavior of body electrolytes, circulatory function etc., is needed.

Significance of Desoxycorticosterone. Because of its availability by synthesis much of the work on this and related adrenal subjects has been based on the use of desoxycorticosterone. This substance, however, has been found in adrenal tissue in only one laboratory (173) and then in minute amounts. The question can be raised, but not answered at present, as to whether it is an adrenal hormone of physiological importance. Whether it is or not affects the conclusions of this paper only as regards the water-retaining aspects of the subject. Salt and water retention, and their sequelae, are brought about more effectively by DCA than other crystalline adrenal hormones, and exaggerated importance might, therefore, be attributed to them by studies in which this steroid was used. Unidentified sodium-retaining materials are present in cortical extracts (95, p. 93) but they are not identical with desoxycorticosterone.

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MOTION SICKNESS

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MOTION SICKNESS is a specific disorder which is evoked in susceptible persons and animals when they are subjected to movements which have certain characteristics. Irwin in 1881 (115) appears to have been the first to use the expression. He suggested that seasickness might more correctly be termed motion sickness "for not only does it occur on lakes and even on rivers, but as is well known, a sickness identical in kind may be induced by various other motions than that of turbulent water, . . ." He thus introduced a convenient and accurate general term, but it did not gain wide use until the beginning of World War II when the late Sir Frederick Banting grouped under the term motion sickness "a variety of conditions akin to seasickness and due to frequently repeated oscillatory movements of the body" (153). While there is some evidence that visual reception of motion may produce a similar sickness in individuals of special susceptibility when their bodies and heads (but probably not their eyes) are motionless (see below), it is questionable whether this response should be classified under the same term, for it is etiologically distinct.

The literature on motion sickness which appeared before World War II contains amazingly little factual information concerning this common disorder. Like the extensive folklore devoted to the subject, most of the publications were replete with uncritical considerations of a number of possible etiological factors and with accounts of uncontrolled and statistically deficient excursions into the field of prevention and cure. The one firm development had been the securing of evidence that the vestibular apparatus is essential for the production of motion sickness. And, subsidiary to this, there was the indication that in ships and boats linear rather than angular accelerations constitute the predominantly effective stimuli. Early in the War, when it became apparent that the moving of large numbers of men by sea and air to and through combat areas might be attended by high rates of motion sickness, the problem began to receive, for the first time, comprehensive experimental attention. Work done during the last seven years has clarified some of the mechanisms involved and has given assurance that a fair degree of control is possible. Much remains to be done. It is hoped that this presentation will stimulate further experimental attacks on a problem which contains many items of general physiological interest.

GENERAL CONSIDERATIONS

Symptoms. The symptomatology of motion sickness is a matter which is important not only for the evaluation of prophylactic and curative measures, but also for

the standardization of procedures used in the experimental production of motion sickness. There are available a number of clinical descriptions of the condition (38, 43, 93, 99, 138). Some early students of the problem attempted to classify cases of seasickness on the basis of the doctrine of vagotonia and sympatheticotonia (52, 99, 101, 139, 236). Thus they distinguished two forms of motion sickness, one in which signs of parasympathetic stimulation are predominant and one characterized by overactivity of the sympathetic system. The soundness of this classification, like the doctrine on which it is based, is most questionable, and recent investigators have discontinued it (5, 94, 175, 209).

Although the symptoms of motion sickness vary somewhat in number and intensity from individual to individual, it can be stated that on the average they are, approximately in the order of their appearance, the following: *drowsiness*, which may mark the transition from a somewhat pleasurable subjective response to motion to one which is suggestive of illness; *pallor*, chiefly facial; *cold sweating*, which appears to be the most reliable and constant indication of the onset of sickness; increased *salivation* with swallowing; the sensation of *nausea*, usually ushered in by some degree of epigastric awareness; and finally *vomiting*. The degree of incapacitation produced varies greatly and may bear little relation to the occurrence of emesis. Headache and 'dizziness' are vague symptoms which do not occur with regularity and cannot be used as specific criteria. It is noteworthy that nystagmus has rarely, if ever, been detected in seasickness and that close observers of the sickness evoked by the motions of airplanes, swings and elevators have failed to find it in their subjects. On the other hand, nystagmus and true vertigo do occur when sickness is produced by rotation.

Blood pressure and heart rate changes are insignificant during the development of seasickness and swing sickness. Some increase in pulmonary ventilation may occur; it is chiefly encountered in highly susceptible persons. No significant changes in blood composition have been found except as the results of other changes (alkalosis after hyperventilation; alkalosis and ketosis after long continued vomiting). Gastric hypotonia and hypomotility are often produced by motion; they occur more often among the susceptible than among the relatively immune, but they do not seem to be invariable accompaniments of motion sickness.

Incidence. The belief that suceptibility to seasickness is confined to a relatively small and special fraction of the population has been dispelled. It is commonly observed that in some individuals a given kind and intensity of motion may produce no symptoms or only mild ones, while in others it evokes severe symptoms. Also, depending on the individual's susceptibility, seasickness may develop within minutes or only after hours of exposure to motion. It appears that a person may be resistant to one type of motion but not to another (30, 61, 65, 95, 138, 149, 179). For example, some tolerate the motions of small boats but become sick on larger vessels, and vice versa. Certain individuals readily become sick on a swing but not in an airplane or ship, and Noble (171) has observed a similar phenomenon in dogs.

It was not until the exigencies of World War II evoked an urgent demand for factual information on this subject that data were collected which indicate with some precision the proportion of the population that is susceptible to motion sickness. It appears that almost every normal individual can be made sick by motion. There are, of course, many influencing factors.

Hemingway and E. L. Green (98) found that during the initial training of student aviators (College Flight Training Program) 11 per cent of 2689 students were sick on one or more of their first 10 flights. The same incidence was found by Rubin (197) in a study of 837 cadets in a primary training detachment. Mathewson (146) reported that of 2682 air crew trainees 15 per cent were sick at least once during their training. McDonough (149) observed that 65 per cent of 380 navigation cadets reported being airsick one or more times during 4534 missions. Similarly, in a study of 1006 experienced flying personnel who were followed after they joined a combat bomber crew training unit, D. M. Green (84) found that during their training 52 per cent of 176 navigator-bombardiers suffered from sickness on one or more occasions and that 23 per cent were sick five or more times. The sickness rates shown by the other categories were: radio-gunners, 32 per cent; navi-bombardiers, 19 per cent; engineer-gunners, 16 per cent; pilots, 13 per cent; armorer-gunners, 11 per cent. The average incidence was 17 per cent. Australian investigators (157) found the incidence of airsickness in navigator trainees to be 33 per cent; persistent sickness occurred in 10 per cent of these subjects. In comparing studies on individuals undergoing primary training with those on navigators, gunners, radio operators and bombardiers, one must bear in mind that during the initial training period the total flying time is short and exposure to rough flying conditions is apt to be avoided because of the inexperience of the students. Although the higher incidence among navigators, bombardiers, gunners and radio operators may have been due in part to the fact that some of these men had been eliminated from pilot training because of airsickness, it is true that their duties require conditions of vision and posture which tend to facilitate the development of motion sickness (see below).

The incidence of sickness has often been reported in terms of 'man missions,' that is, the amount of sickness found in, say, 50 men during 100 flights (5000 man missions). Hemingway and E. L. Green (98) reported 661 cases of airsickness among 2689 men, each of whom took 10 flights, an incidence by flights of approximately 2.5 per cent. Brown, Brett and Howlett (45) found an incidence of 8.3 per cent during 2775 manflights by 91 navigation students. On the basis of man missions, McDonough (149) reported an incidence of 15.6 per cent among 380 navigation cadets who flew 4534 missions. In a Navy study (127) an incidence of 7.5 per cent was found in 531 cadet flights. When the trips were rough, as was the case in 205 of these man missions, the incidence was 25 per cent. The practice of reporting sickness rates on the basis of man missions demonstrates the magnitude of the problem for the armed services, but it masks the true situation, since the effects of adaptation and the influence of different degrees of roughness during different flights are not indicated.

In an airborne maneuver involving 10,070 men on a flight of 130 to 150 miles under ideal flying conditions the incidence was 3.56 per cent (147). In a study (131) of the effects of preventives during airborne maneuvers it was found that the incidence of sickness among the untreated controls varied in general with the length of the flight; it was concluded that "in flights exceeding two and one-half hours and performed under normally-expected variations of flying conditions, more than one quarter of the passengers may be expected to become airsick." Littauer (168) reported an incidence of 80 per cent in a five-hour flight involving airborne troops in planes and gliders. Park (180) found an average incidence of 35 per cent during glider maneu-

ers. In an experimental study (221) of airsickness, 65 unselected but experienced parachutists were exposed to 'moderate evasive tactics.' After 20 minutes all 65 subjects, together with 4 observers and 2 radio operators, were airsick.

As regards seasickness, Hill (99) estimated that over 90 per cent of inexperienced persons will become ill under severe conditions. The correctness of this estimate is borne out by more recent studies. British investigators reported incidences of seasickness in small boats ranging from 15 to 70 per cent (103, 105, 107). In tests of the efficacy of pharmacological preventives Tyler (220, 221) obtained data on sickness rates in small landing craft during amphibious training operations which lasted from one to three hours. In the 36 groups, totaling 3133 unselected young men, which received placebos (lactose in capsules) the rates varied from 11 per cent, when there were gentle swells, to 60 per cent, when the seas were moderately rough. In the former instance only one-fifth of those affected were severely sick (severe nausea with or without vomiting), in the latter, one-half. Since, in the course of these same observations, it was found that the giving of a placebo did not affect the incidence or severity of sickness, these rates may be regarded as indicative of the general level of susceptibility to the kinds and degrees of motion experienced. They suggest that unofficial reports of rates approaching 100 per cent in certain combat landing operations during the War were not exaggerated.

In many experimental studies of motion sickness, swings have been used to test susceptibility and the effects of drugs and to explore other aspects of the problem. Parker, Fields and Sellers (184) reported that 36.4 per cent of 2950 men showed symptoms during a swing test of 30 minutes. Hemingway (94) found that of a group of 489 normal young adults exposed to the motion of a swing for 20 minutes (or less, if vomiting occurred) 29 per cent became sick. In a study (95) of 1500 men in the Army Air Forces, whose airsickness histories were known, this brief swing test gave vomiting rates which varied from 11.3 per cent for flyers who had never been airsick to 65.4 per cent for airsickness eliminees. Noble (173), using a somewhat more severe swing test, obtained rates of vomiting as high as 56.6 per cent.

Individual Predisposition and Adaptation. It has been pointed out above—and it is common knowledge—that there are great variations among individuals in susceptibility to motion sickness. Failure to control this factor in experimental studies has resulted in some errors. In such work it is essential to use either carefully selected subjects of known susceptibility or, where this is not possible, large heterogeneous groups randomly divided into the necessary number of sub-groups.

It has been stated that children under 2 years of age and elderly persons show less susceptibility than those of intermediate age (30, 38, 43, 44, 61, 114, 178). Some indication was obtained by Noble (173) that susceptibility to swing sickness increases after the age of 40. It has been held, though on the basis of scanty evidence, that the incidence is higher in females than in males, and that there are differences between races (38, 43, 44, 54, 178). Many deaf-mutes, and persons with internal ear damage are immune (117, 196, 162, 205, 47). It has long been held that 'neurotics' are more prone to motion sickness than 'stable' individuals, but there is actually very little evidence to support this rather popular view (see below). A number of writers (60, 71, 79, 99, 176) have claimed that individuals characterized by an abnormal excitabil-

ity of the parasympathetic system (the 'vagotonia' of Eppinger and Hess) are particularly susceptible. Some apparently favorable results obtained with atropine medication (together with a disregard of the central effects of this drug) constituted the principal support for this idea.

There is evidence that horses, dogs, cows, sheep and chickens are subject to motion sickness (52, 73), and that rabbits, guinea pigs and pigeons are immune (52, 121, 192, 218). Dogs show degrees of susceptibility that are about the same as those displayed by the human race (170, 171), but cats and monkeys are highly resistant (22, 164, 171).

A good many attempts have been made to discover the physiological or psychological peculiarities of an individual which determine his degree of susceptibility to motion sickness. Actually little has been learned of the basic factors which underlie vulnerability. Scattered through the pages that follow are references to a number of opinions, suggestions and a few experimental facts which bear on this important aspect of the problem. By way of summary about all that can be said is that susceptibility appears to depend on a rather specific constitutional capacity to respond to certain patterns of vestibular stimulation and that it can be modified to some extent by several extralabyrinthine influences.

It is estimated that about 95 per cent of all susceptible persons are capable of adaptation to motion. Hemingway (97), in a study of 198 aviation students, found a progressive decrease in the incidence of airsickness during the training period. Eighty-four per cent were sick during the first flights, only 10.5 per cent on the 10th flight; the decrease was more rapid in the first than the second 5 flights. Joekes (120) reported that while the incidence of airsickness was 17 per cent during the first few hours of flying it dwindled to 0.5 per cent after 10 hours of experience in the air. Adaptation also occurs on swings. In a study of 254 air gunners, Joekes found that although the incidence of sickness during a first swing session was 20 per cent, only 2.8 per cent of those who were sick failed to adapt during subsequent swinging. Manning (141) divided 100 unselected subjects into two groups. The members of one group were swung for 15 minutes on each of 10 successive days; the members of the other group were not swung until the 11th day. On the 11th day the first group showed an incidence of 18 per cent, the second one of 42 per cent.

In spite of some contradictory reports, the evidence available indicates that adaptation to one type of movement does not necessarily adapt the individual to the motion produced by a different vessel, airplane or experimental device. Gibson Manning and Cohen (81), in a study of 150 student air navigators which involved 4000 swing sessions and 1250 flights, found that daily exposures of 15 minutes to the motion of a swing for two weeks before and during the flying period did not reduce the amount of airsickness.

It appears that the capacity to adapt is minimal or entirely absent in those highly susceptible individuals who make up perhaps 3 to 5 per cent of the population. Brown, McArdle and Magladery (47) secured evidence that while some very susceptible subjects develop an increased tolerance when repeatedly exposed to a swinging motion, such adaptation does not occur in chronically sick individuals. Morton and McEachern (165, 166) attempted to produce adaptation in a very susceptible

flyer who had been grounded because of recurring airsickness. As a result of daily exposures to motion for 23 days there developed only a small degree of tolerance; it was insufficient to enable him to return to flying duty.[1]

Experimental Methods of Producing Motion Sickness. The means which have been effectively used to produce motion sickness experimentally are: *a*) devices which reproduce artificially the movements of a ship; *b*) elevators or elevator-like machines; *c*) swings; *d*) apparatus which subjects an individual simultaneously to rotation and tilting movements of the head.

Quix (195) cites the early work of Kreidl, who apparently produced motion sickness in animals by artificial ship movements. Pozerski (191) designed a see-saw-like machine that simulated the pitch and roll of a ship. In his extensive studies Sjöberg (204, 205) first used ordinary passenger elevators (lifts). When he found that "strong acceleration and a large amplitude" were required to produce symptoms quickly and easily he constructed a crane. The 'Roll-Pitch Rocker' constructed at the Montreal Neurological Institute in 1942 (56, 165) and the similar but smaller machine built at the Banting and Best Department of Medical Research in Toronto were designed to reproduce the movements of a ship at sea. Although they proved effective they later gave way to simple swings which permitted the ready testing of larger numbers of subjects. Wollaston (232), who was apparently one of the first to try the swing in a study of motion sickness, stated as early as 1810 that "the sickness occasioned by swinging is evidently from the same causes as sea-sickness. . . ." The first to use the swing extensively for experimental purposes were workers in Russia (234, 235) and Brown, McArdle and Magladery (47) in England. It was soon adopted by various workers in Canada, Australia and the United States. An advantage of the swing and also of the elevator designed by Wendt (5, 225) is that, since the various characteristics of the motion can be controlled, large numbers of subjects can be exposed to identical conditions of motion. The types of swings generally employed are the 2-pole and the 4-pole; both may be operated manually or electrically.

The 'wave machine' of Wendt (5, 225) is a hydraulically driven elevator with an air-tight, sound-proof, temperature-controlled cab, which operates in a shaft allowing vertical excursions up to 18 feet. It is essentially a vertical accelerator in which velocity, acceleration level and amplitude can be varied to give a variety of wave forms. It was designed primarily for the study of the characteristics of a motion which make it nauseating. It has proved capable of inducing severe sickness.

Many workers, impressed by the similarity between disturbances produced by rotatory movements and those produced by moving vessels, have employed rotating

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[1] There is evidence that, as regards adaptation, dogs are quite like human beings. A few instances of this phenomenon were encountered by one of us (26) in dogs which were subjected, at weekly intervals, to the motion of a swing, but the only substantial study of adaptation in animals of which we are aware came to our notice while this review was still in progress. Professor R. L. Noble of the University of Western Ontario very kindly provided us with a copy of the manuscript of his paper entitled "Adaptation to Experimental Motion Sickness in Dogs" (170a). This report makes it clear that adaptation is produced readily in animals having a relatively low initial susceptibility, but not easily in highly susceptible dogs. The rate of the development of the refractory state appears to be proportional to the frequency of exposure and perhaps to the duration of the individual exposures. It is interesting to note that adaptation occurred even though the animal was effectively treated by the thiobarbiturate, V-12.

chairs, tables and the like. For the production of sickness the subject must be exposed to repeated accelerations and decelerations. When an individual is rotated in the usual way on a Bárány chair changes in velocity occur only at the beginning and the end of rotation. As will be disclosed below, the essential repetitive changes in velocity exert their effects through non-auditory labyrinthine receptors. When rotation occurs with the head fixed, the spatial relationship of the various parts of the labyrinth to the axis of rotation remains constant, a fact which has been emphasized by Spiegel (216). Actually this type of motion is not very effective in producing sickness. Symptoms of much greater intensity have been obtained by changing the position of the head during rotation. For an experimental study of motion sickness, Spiegel, *et al.* (216) devised a chair in which, with each turn, the head of the subject is tilted either in the sagittal or the frontal plane. Thus the angle between the plane of rotation (horizontal) and the plane of the horizontal or vertical semicircular canals is made to increase and decrease with each rotation. This exerts the same effect on the canals as would repetitive changes in velocity. The apparatus proved to be very effective in producing sickness. Spiegel *et al.* believe that this device subjects the labyrinth to a type of stimulation which is essentially the same as that provided by other effective forms of motion. It is certain, however, that its effects on the labyrinth as a whole and on the utricular maculae in particular are quite different from those produced by a swing, an elevator, a plane or a vessel. The emphasis in the work with this apparatus is on stimulation of the cristae, but the authors suggest that stimulation of the maculae may play a role in the genesis of the sickness obtained.

ETIOLOGY

It was the opinion of Hippocrates that when a draught of hellebore had been given to provoke emesis the patient "should be made to move more about, and indulge less in sleep and repose" (*Aphorisms, IV, 14*; ref. 3). He supported this precept by stating that "sailing on the sea shows that motion disorders the body." It is evident that the Father of Medicine believed that seasickness is caused by the movements of the ship. Some recent writers, especially those with psychiatric interests, have not agreed with this view and have been inclined to attribute both seasickness and airsickness to other disturbances than motion. The occasional failure to appreciate the fact that the fundamental cause of motion sickness is motion has led to some confusion and conflict of ideas. It is perhaps explained, at least in part, by the fact that a stimulation of labyrinthine receptors, adequate to evoke motion sickness, produces little effect on consciousness. As Hemingway (96) has pointed out, "the patient is sick without being aware of the cause" and therefore is apt to attribute his troubles to odors, overheated compartments, visceral sensations, disagreeable sights, distasteful food or anything else unpleasant, unusual or uncomfortable which he experienced shortly before or simultaneously with exposure to motion. Unfortunately some observers have adopted one or another of these interpretations of their subjects.

Characteristics of Effective Motions. From a logical point of view the first step in an analysis of the cause or causes of motion sickness should be the determination

of those characteristics of motions which make them nauseating. Common observation suggests and experiment demonstrates that an essential feature of an effective motion is a repetitive change in velocity.

One group of early students of the problem, influenced by knowledge of the nauseous effects of rotatory movements, were inclined to attribute seasickness to angular accelerations in the movements of ships. Others made a careful examination of these movements and concluded that in the production of *mal de mer* linear accelerations in the vertical plane are prepotent. A majority of both groups, aware that destruction of the labyrinths abolishes all susceptibility to motion sickness, were primarily interested in applying their conclusions to the resolution of the question whether the receptors involved are the otolith organs or the cristae of the semicircular canals (see below). These earlier studies did not contribute any precise information concerning the essential features of effective movements. Only recently, as a product of the widespread interest in motion sickness engendered by the events of World War II, has some definite knowledge of this aspect of the problem been secured. The chief results have been obtained by the experimental determination of the effects of altering certain characteristics of the motions of swings and vertical accelerators.

Cipriani (55) demonstrated that when a subject is seated upright on a swing, radial acceleration acting in the plane of the long axis of the body is the principal vestibular stimulus. He points out that swinging also involves three main components of tangential acceleration. The largest, that relative to objects around the swing, affects equally the mobile and the fixed portions of the vestibular apparatus and therefore cannot serve as a stimulus. He describes two other tangential accelerations which may be slightly effective stimuli. It is of interest to note that the *g* changes acting on the head of an individual subjected to the motion of swings used in experimental studies of motion sickness are of approximately the same order of magnitude as those which occur in the vertical direction in a certain type of plane (Anson) flying in moderately rough air (46).

In the case of a swing the magnitude of the maximal change in *g* depends on the arc; as long as the angle through which the swing oscillates remains constant, a change in radius does not alter the magnitude of the radial (linear) acceleration. On the other hand, the radius determines the frequency of oscillation and the duration of the change in velocity; the shorter the distance from the fulcrum, the greater the frequency. Thus if the arc be kept constant one may vary the frequency of occurrence of the same *g* change by altering the radius.

The effects of altering either frequency or maximal change in *g*, while keeping the other factor constant, were investigated by Fraser and Manning (78) in a study of 250 unselected normal individuals (aircrew personnel at an Initial Training School). Each subject was swung for 30 minutes unless extreme nausea or vomiting or both necessitated stopping before that time. In one series of experiments the rate of swinging was kept constant at 17 per minute while the maximal change in *g* was varied by changing the arc. With a maximal change of 0.25 *g* the incidence of sickness was 22 per cent; increasing the *g* change to 0.9 more than doubled the sickness rate (50%), but a further increase to 1.7 *g* resulted in an incidence of 46 per cent. Somewhat simi-

lar results were obtained by Noble (171) in experiments on dogs. Using a constant radius giving a frequency of 14.5 per minute he found that arcs of 22.5°, 45°, and 90° produced sickness rates of 37, 90 and 100 per cent, respectively. These two sets of results suggest that increasing the *g* change increases the effectiveness of the motion up to a certain point beyond which further increases fail to augment the incidence. Changes in the frequency of a swing appear to exert similar effects. When Fraser and Manning (78) kept the *g* change constant at 0.9 and varied the radius they found that although a decrease in frequency from 22 to 17 per minute increased the incidence from 4 to 50 per cent, a further slowing to 15 merely raised the sickness rate to 58 per cent. In his dog experiments Noble secured evidence that for any given angle of swinging there is an optimum frequency (radius). Increasing or decreasing the frequency caused a falling off in the incidence of vomiting. The only exception occurred when the arc was small (22.5°); then the sickness rate showed a second rise when the frequency was greatly augmented by making the radius very short (37.5 inches).

The motion of a swing is made up of a vertical, a horizontal and a small angular component. Noble (171) separated these components and subjected dogs of known susceptibility to each. The horizontal proved to be the most effective, the vertical induced vomiting only in the more susceptible animals, and the angular failed to provoke any signs of sickness in the most susceptible dogs. It is significant that the composite motion was found to be more effective than any one of its components. McIntyre (156), who used human subjects, found that the repetitive up-and-down motions of an elevator, with accelerations only in the vertical plane, produce less sickness than do swings which yield comparable *g* values (see also 142). He also found that while the angular components of the motion of a swing are by themselves not nauseating, the addition of small angular components to movements in the vertical plane greatly increases the effectiveness of the latter. Thus we are faced with the probability that the repetition of a combination of linear accelerations in different planes or of linear and angular accelerations may constitute a more potent stimulus than the repetition of any one acceleration.

Work done by Wendt and his colleagues (5–11) constitutes the most extensive attack yet made on the problem of determining those features of a motion which make it nauseating. These investigators used the 'wave-machine' described above and thus confined their analysis to movements in the vertical plane. Their subjects were 477 Naval Aviation cadets. Exposure was for 20 minutes unless vomiting occurred earlier. The subjects sat blindfolded, with head in the upright position and with the cab temperature at 86° F. Evidence of the careful design of the experiments will be found in the original papers.

Wendt was led to examine the effects of varying the time interval between accelerations by the common observation that vehicles and movements which yield large accelerations with brief phases are rarely nauseating whereas those having low accelerations and long phases are apt to be very productive of nausea and vomiting. The first test (5) of the hypothesis thus suggested was a comparison of the effects of four waves in which the interval between equal accelerations at top and bottom was made to vary by changing the duration of a period of constant velocity (400 ft/min.) inserted in the middle of each wave. In the different waves this period was 0.22,

0.68, 1.12 and 1.6 seconds, the cycles per minute, 32, 22, 16, and 13, and the amplitudes, 4, 7, 10 and 13 feet. As the temporal separation of the accelerations was increased the subject was exposed to fewer and fewer of them and less total work was done on him. The 16-cycle wave with only half the rate of power expenditure but twice the duration of the 32-cycle wave produced six times as much sickness. The other waves were intermediate in effectiveness. Evidently the wave of longest duration (13 cycles) represents a recession of the optimum condition. These results confirmed "the hypothesis that the time characteristic of a motion rather than its violence is the feature relevant to motion sickness."

A second investigation (6) of the same hypothesis dealt with the question whether the significant variable is total wave duration or duration of the period between accelerations. Three wave types were obtained by changing not only the period of constant velocity in the middle of the wave but also the velocity itself (the greater the duration the less the velocity) while holding the wave cycle duration constant at approximately 22 cycles per minute. Peak values of acceleration were held constant. The intervals between accelerations were varied by varying the length of time of the application of the acceleration. The total energy per cycle (measured by peak velocity) increased as the interval between accelerations decreased. It was found that an increase in time between accelerations from 0.68 to 1.12 seconds with a reduction in total energy per cycle, resulted in a significant decrease in sickness. It was therefore concluded that when total wave duration is held constant, the total energy per wave is a more potent factor than is the interval between accelerations.

A third study (7) was devoted to a determination of the effects of acceleration level. Four wave types were obtained by controlling the rate of change of velocity and so altering the frequency and the total wave amplitude (between extremes of 4 and 9 ft.). The total energy per wave was kept at a constant value (mid-wave velocity of 400 ft/min.). The fastest wave with the highest acceleration level (32 cycles, 0.65 *g*) produced the least sickness (13%) while a wave of moderate frequency and moderate acceleration (22 cycles, 0.36 *g*) gave maximum sickness (53%). Two slower waves at lower acceleration levels (16 cycles, 0.25 *g*; and 13 cycles, 0.20 *g*) were somewhat less effective (rates of 43 and 40%). The results showed that nausea and vomiting are most effectively evoked by waves of moderate frequency and acceleration level. As regards the effects of frequency and *g* value they are in accord with those obtained by Fraser and Manning (78) in a study of the characteristics of the motion of a swing which make it nauseating.

The purpose of a fourth study by Wendt and his collaborators (8) was to find whether sickness production is a function of wave frequency or of duration or magnitude of component accelerations. Five waves were used, all having the same total energy. One, like the waves previously described, was a symmetrical acceleration wave. Four were asymmetrical; the amplitude, duration and *g* value of the acceleration differed in the top and bottom halves. The cycle duration of two of the asymmetrical waves was the same as that of the sickness-producing symmetrical wave used, while that of the other two was almost identical with the duration of the most nauseating wave of the third study. Consequently all the asymmetrical waves had intervals between accelerations which might be expected to induce high sickness rates

if cycle frequency is important. The top and bottom halves of the unequal waves had different acceleration levels. In two the different *g* values were ones which when used in symmetrical waves were relatively non-nauseating. Each of the other two was composed of a relatively ineffective half-wave and a nauseating half-wave. The sickness rates obtained were very close to what would be expected if the half-wave character rather than wave frequency determined the result. The authors indicate that this conclusion infers that effective motion acts on some resonant mechanical system with a natural period of about 3 or 4 seconds, but they point out that all structures in the body likely to be involved are short-period, heavily damped systems. They recommend therefore that acceptance of the conclusion be deferred until further evidence is forthcoming.

Vestibular Factors[2]. The idea that motion sickness is due to vestibular stimulation probably had its origin shortly after Goltz (1870), and Breuer, Mach and Crum Brown (1873–74), by elaborating the much earlier observations of Purkinje (1820–25) and Flourens (1828), showed that motion evokes responses through activation of labyrinthine receptors. According to Sjöberg (205), Irwin and Palasne de Champeaux were probably the first (1881) to call attention to the similarity between seasickness and Menière's disease and to suggest that the former is caused by stimulation of the labyrinth. This conjecture gained support from early observations which indicated that many deaf-mutes and others with internal ear damage are immune to seasickness. In 1882 William James (117) reported that approximately one-third of 519 deaf-mutes he had studied were not made dizzy by rotation, and a little later Pollack (188) found that he was unable to provoke vertigo by galvanic vestibular stimulation in 30 per cent of the deaf-mutes he examined. A majority of the refractory subjects studied by James suffered from acquired deaf-mutism. Fifteen of them had at one time or another been exposed to rough weather at sea, but not one had been sick. A few years later Minor (162) mentioned observations made at sea which indicated that many deaf-mutes are not susceptible, and Reynolds (196) pointed out that persons with internal ear damage produced by otorrhea are immune to seasickness. Much later Sjöberg (205) reported that 3 girls from a school for the deaf, who showed no responses to severe caloric and rotational vestibular stimulation, were not made ill by exposures of 2 hours to the motion of an elevator, although this same stimulus caused sickness within 30 minutes in most normal individuals. Still more recently Brown, McArdle and Magladery (47) found that 5 patients, whose labyrinths had been destroyed by meningitis, neither exhibited nor experienced any sign of sickness during 40 minutes of swinging.

Quix (195) states that Kreidl was the first to produce experimental motion sickness in animals; he exposed them to artificial ship's movements. This worker, according to several writers (54, 61, 193, 195, 205) found that after bilateral section of the eighth nerves the animals were insensitive to motion. Since Kreidl apparently

[2] In 1942 McNally and Stuart (159) published an excellent review of the physiology of the labyrinth which set forth all available relevant information that might be useful to the student of motion sickness. In this section we have drawn heavily on their presentation, but we have sought to limit our considerations of the functions of the non-auditory labyrinth to those items which seem to bear most directly on the problem of the etiology of motion sickness. We include a number of experimental facts and certain theoretical points which have been put forward since 1942.

never published the results of these experiments, the credit for first proving experimentally the indispensability of the labyrinths in the production of motion sickness goes to Sjöberg whose classical work reported briefly in 1929 (204) and fully in 1931 (205) demonstrated that bilateral labyrinthectomy renders susceptible dogs wholly immune to motion sickness. Before the operation his 4 dogs had shown marked symptoms and had vomited after 11, 12, 20 and 30 minutes when exposed to the motion of a crane. After the destruction of the labyrinths they failed to develop any sign or symptom of motion sickness during exposures of 3 hours. Recently McNally, Stuart and Morton (160) and Babkin and Bornstein (20) have confirmed this important discovery.

A question which has been and remains controversial is whether one or more than one of the sense organs of the non-auditory labyrinth discharge the afferent impulses which are essential for the production of the symptoms of motion sickness. There are three schools of thought on this question: One believes that both groups of receptors, the cristae of the semicircular canals and the maculae of the otolith organs, are involved; another school maintains that only the maculae are concerned; a third group holds that the otolith organs are not essentially involved and that motion sickness is caused chiefly, if not solely, by stimulation of the cristae.

It appears that the only labyrinthine sense organs to be considered in this connection are the cristae of the semicircular canals and the utricular maculae. Although the saccule has been considered by many to be part of the vestibular mechanism, the observations of a number of workers strongly indicate that it is not concerned in the elicitation of any known labyrinthine static or kinetic reflex but may be responsive to vibrations. (A full and convincing discussion of this work is given by McNally and Stuart, 159.) Thus a considerable weight of evidence seems to eliminate the saccular maculae as receptors involved in the genesis of motion sickness.

Although Brown (48), Magnus and de Kleyn (137) and de Kleyn and Versteegh (123) believed that linear and progressive movements stimulate the semicircular canals, it is now the consensus of opinion that the utricular maculae are the receptors chiefly stimulated by linear accelerations and that the sense organs of the canals respond chiefly to angular accelerations. However, there is some evidence that the utricles can be stimulated by very rapid angular rotations (159). Nystagmus is a result of stimulation of the cristae. Neither stimulation nor injury of the utricle produces it (88, 137, 223, 224). A depressor response to rotation can be evoked after the cristae have been made inexcitable by puncture of the round window, but it can also be evoked after all known reflexes of utricular origin have been paralyzed by the centrifugation method, provided the semicircular canals remain functionally intact (129). It has been concluded from such studies that the non-auditory labyrinth functions as a whole and that its two types of receptors can substitute for one another.

Many of the earlier workers (1, 2, 24, 50–52, 138, 199), impressed by the similarity between the symptoms experimentally produced by angular accelerations and those of seasickness, arrived at the conclusion that the semicircular canals are partly, or even wholly, responsible for the production of seasickness. Bruns (50, 51) found that persons who were highly susceptible at sea were easily made sick by rotary movements, while those who were immune on shipboard were relatively insensitive to the nauseating effect of rotation. Fischer (72, 73) also believed that the semicircular

canals are responsible, since in his experiments typical nausea was produced by rotation. He held that stimulation of the maculae is not necessarily involved in the genesis of seasickness. He evoked severe symptoms by rotating his subjects in a chair and simultaneously having them move their heads with an even rolling motion so that the position of the head was constantly changing in relation to the axis of rotation. He reported that under these conditions there was no distinct nystagmus. Spiegel and his associates (216) obtained high rates of sickness by means of their rotating-tilting machine (see above). In these experiments nystagmus always occurred. Almost all observers agree that nystagmus is not observed in seasickness, airsickness and swing sickness (42, 63, 108, 138, 169, 194, 205, 229). Humphreys (114), however, in listing the symptoms of seasickness stated that "temporary nystagmus has even been observed," but he gives no further information on the point. In considering the sickness produced by angular accelerations it should be borne in mind that several careful studies have failed to establish any good correlation between responses obtained in the Bárány chair and susceptibility to airsickness or swing sickness (see below).

The absence of nystagmus in seasickness has been emphasized by many as an indication that the receptors of the semicircular canals are not stimulated by the movements of ships and hence are not concerned in the genesis of seasickness. Wojatschek (229) concluded that angular accelerations do not, in the case of a rolling ship, have an effect because the phases of motion succeed one another rapidly and so annul one another. Sjöberg (205), who concluded that in seasickness both the semicircular canal system and the otolith apparatus are effectively stimulated, suggested that the absence of nystagmus is due to stimulation of both labyrinths in such a way that the impulses from one side counteract the effects of those from the other side and therefore no observable eye movements occur. But since Fleisch (76) had reported that in rabbits small reflex eye movements, not detectable by the naked eye, can be evoked by horizontal linear acceleration, Sjöberg thought it highly probable that similar responses, not observable by ordinary inspection, occur during experimental exposures to linear accelerations in the vertical plane and on shipboard when the waves are high. Maitland (138) contended that the absence of nystagmus in seasickness can be explained on the basis of the time periods of the various movements of the ship, particularly the pitch, which is the most nauseating. With Major Reed he determined that one phase of this movement rarely exceeds 5 seconds, which is only one-third of the time taken by the 8 rotations of the chair test. Further, he claimed that the pitch acts on the vertical canals rather than the horizontal. Therefore the comparison should be made between the effect of the ship's motion and a chair test of three continuous rotations which act on the vertical canals, a stimulus which is unlikely to elicit the primary reactions such as nystagmus. Spiegel and his co-workers (216) hold that nystagmus is not produced in the seasick because the rolling movements of a ship have a relatively small amplitude, each being in the direction opposite that of the preceding one, and are not complete rotations through 360 degrees in any one direction. According to these authors this results in only small oscillations of the cupola insufficient to cause ocular reflexes, but strong enough to produce 'vestibulo-vegetative' reactions.

Indirect evidence in support of the theory that the utricular receptors are the

sense organs involved in the production of seasickness has been derived from studies of the types of movements produced by ships. It has often been stated that the vertical movements of a ship are more distressing than the rolling movements. Wilks (226) first pointed out the similarity of the sensations produced by the pitch of a ship and those evoked by an elevator. Wojatschek (229), Maitland (138), Quix (195), Sjöberg (205) and others (44, 163) made extensive mathematical analyses of the movements of ships. On the basis of threshold values of labyrinthine stimulation by angular and linear accelerations all concluded that the linear (horizontal and vertical) rather than the rotatory or angular movements are the principal stimuli in the production of seasickness. As early as 1875 Mach (see 159) had calculated the thresholds for stimulation by both angular and linear (vertical) accelerations. He estimated that for the former it is 2 to 3° per second acting for about 15 seconds, for the latter about 0.01 *g* (approximately 10 cm/sec.). Although Quix (195) cites others who give different values for angular acceleration, Mach's figures are still considered by many as standard. Both Quix and Sjöberg concluded that linear accelerations produced by the movements of a ship are well above the threshold for stimulation of the maculae and are, therefore, responsible for seasickness. They found that the angular accelerations are well below the threshold of stimulation of the cristae. Wojatschek (229) on the other hand, determined that in the movements of a small boat with a roll of 60° (which is an extreme roll) the angular accelerations are above the threshold value, but he concluded that they are not important in the elicitation of sickness. According to Maitland (138) any statement that the rolling of a large vessel exceeds 30° should be received with caution. In view of the information now available concerning the characteristics of motions which make them nauseating (see above), it is evident that considerations of the mere threshold of simple reactions or sensations evoked by labyrinthine stimulation have little significance for this problem.

Several authors (17, 116, 153, 200, 205) refer to work done with a suspended cabin designed to eliminate angular accelerations in the movements of a ship. It is significant that passengers in such a cabin, still exposed to the vertical movements, become seasick. Also, it is now well established that motion sickness can be readily provoked by elevators and other devices which yield only vertical accelerations.

The recent discovery that the incidence of swing sickness is enormously affected by the position of the head, regardless of the position of the body, gives further emphasis to the role played by utricular stimulation in the etiology of motion sickness. Quix (195) pointed out that the position of these receptors is such that they would be particularly responsive to linear vertical motion. He suggested that differences in sensitivity to motion could be brought about by altering the head posture and hence the relation of the otolithic apparatus to the plane of linear motion. The results of studies of swing sickness made recently by Howlett and Brett led to an analysis (111) which has indicated that the utricular maculae are responsible for this form of motion sickness. This important aspect of the problem is further discussed below.

It is perfectly clear that motion sickness can be produced by either angular or linear accelerations. When it is evoked by the former, the semicircular canals appear to be primarily involved and nystagmus is observed; when it is produced by vertical

or other linear accelerations the utricles are stimulated and no perceptible nystagmus occurs In practically all cases of sickness due to the motions of ships, boats, airplanes, elevators and swings, linear accelerations appear to constitute the principal stimuli. It is to be emphasized, however, that when it is said that the effective accelerations produced by ships and airplanes are ***principally linear***, this does not mean that angular accelerations are absent or wholly ineffective. It is well known that the most disturbing movement of a ship is the 'corkscrew' or 'wobble,' a combination of scending (vertical accelerations) with pitching and rolling (angular and linear accelerations combined). When the passengers of an airplane are exposed for a short time to this sort of composite motion, as when 'mild evasive tactics' are executed, extremely high rates of sickness result (221). In this connection the observations of Noble and of McIntyre (described above) are of significance. They indicate that the addition of small angular accelerations to a motion which has only linear changes in velocity augments its nauseating properties. Since it is most unlikely that the angular accelerations which were added could have stimulated the utricle, they probably acted through the semicircular canals.

Visual Factors. The idea that the eyes are involved in the genesis of motion sickness is an old one. Irwin (116) points out that in his *Zoönomia* (1794) Erasmus Darwin, whose grandson suffered so greatly from the motions of the 'Beagle,' expressed the view that visual disturbances constitute the principal cause of seasickness.

It seems to be fairly well established that, in the absence of any motion of the head and body, visual reception of motion may produce some of the visceral changes of motion sickness (58), nystagmus and loss of equilibrium (41) and even nausea and vomiting (153). In the 'witch's house' of amusement parks a visual influence, by seeming to accentuate a slight motion, frequently causes sickness (62, 153). Sjöberg (205) in exposing 4 dogs to the movements of his crane found that the onset of symptoms was somewhat delayed when the eyelids were sutured. It is known that blind individuals may become motion sick, but it has not been determined whether the incidence of sickness among the sightless is different from that found among normal individuals. It has been reported that the showing for more than 30 minutes of a motion picture taken from the subject's seat in a moving swing failed to produce sickness in a group of students seated in a classroom (165).

Although, as just indicated, visual stimuli may facilitate the production of sickness by motion or even provoke it in the absence of labyrinthine stimulation, visual influences are more apt to ameliorate the effect of motion. In many of the recent studies of the sickness produced by swings and elevators care was taken to control visual factors by blindfolding the subjects. The results of at least 4 series of experiments (135, 143, 144, 144a, 157) have shown that susceptibility to swing sickness is greater when vision is excluded, either by blindfolding or by enclosing the swing.

A common observation is that when aircraft pilots fly as passengers they tend to show an augmented susceptibility to motion sickness. It is held that an important factor in the production of this change is the absence of the visual orientation which is possible in the pilot's seat (19, 75, 84, 190). The relatively high incidence of airsickness found among aerial navigators and radio operators (84, 91, 93) and of seasickness among troops crouching below the gunwales in landing craft (220, 221) also

appears to be due in large measure to loss of visual contact with the ground or the horizon.

Spiegel and his co-workers (216) in studying the responses of 30 individuals to the motions of their rotating-tilting machine found that the incidence of sickness tended to be less when the subject fixed his gaze on a lamp which participated in all the movements of the head than when he watched 3 stationary lamps placed around the rotating chair. It is not easy to relate this observation to those mentioned above, but attention may be called to the possibility that the very rapid and complex eye movements which must have attended the 'watching,' but not the 'fixation,' constituted a factor of importance.

Role of Non-labyrinthine Proprioceptive Stimulation. Stimuli belonging to this category may influence the production of motion sickness. Poppen (189), in discussing the concept that seasickness is a disability of equilibration, alluded to the fact that, after blindfolding, equilibrium is quite well preserved in an individual with non-functional labyrinths, while exclusion of vision in a tabetic causes serious disturbances of equilibrium. From these well known facts he concluded that somatic changes are important in the genesis of motion sickness. The evidence now at hand suggests that body movements and body position as such are of little significance in the production of motion sickness. To be sure, Sjöberg (205) found that encasing his dogs in plaster casts lengthened somewhat the time before the symptoms of motion sickness appeared, an observation which indicates the desirability of examining further the role played by non-labyrinthine proprioceptive stimulation in the genesis of motion sickness.

In 1931 Flack (74) provisionally concluded, as a result of observations made at sea, that "generally speaking, subjects in whom eye muscle imbalance is not induced or aggravated do not suffer from sea-sickness. . . ." Best and his co-workers (31) found a definite increase in ocular imbalance after swinging, especially in susceptible individuals. On the other hand, Howlett and Brett (109) have reported that ocular muscle balance was poorer in a non-airsick group than in a group composed of persistently airsick trainees. The available data do not seem sufficient to permit any conclusion regarding a possible relationship between heterophoria and susceptibility.

In the face of the evidence that head position (i.e., the position of the labyrinthine receptors), regardless of body position, is such a prepotent determinant of the incidence of motion sickness all extra-labyrinthine stimuli appear to fade into relative insignificance.

Effect of Position. Much light has been thrown on the problem of the etiology of motion sickness by studies of the effect of altering the position of the individual in relation to the motion or motions to which he is subjected.

Awareness that the recumbent posture has a beneficial effect on the symptoms of seasickness is as old as navigation itself. Contributors to the early medical literature on the subject gave many, and sometimes bizarre, reasons to account for this effect. The more reasonable writers, in those days, tried to explain the phenomenon on the basis of the effect the horizontal position might have in reducing gross movements of the heavy viscera with tugging on nerve plexuses. Authors who were adherents of the ideas of Wollaston (see below) attributed it to possible circulatory alterations. Even today there are those who offer these explanations or modified versions of them.

It appears that Wilks (226) was the first (1875) to suggest that the ease with which seasickness is produced in the vertical position (as compared to the horizontal) may be related to the vertical motion of the ship. He arrived at this conclusion by noting that symptoms similar to those of seasickness could be produced in passenger elevators (lifts), which were being introduced at about that time. Irwin (115), impressed by the early work of Purkinje, Breuer, Mach, Crum Brown and others, was the first to offer an explanation based on the effect the assumption of a horizontal posture has in altering the position of the head, and thus that of the labyrinth. The relation of the position of the head to the incidence of seasickness received attention in the writings of Byrne (52), who found that symptoms typical of seasickness are much more easily produced on a rotating table when the subject is sitting with his head in a central position as regards the axis of rotation than when he is in the supine position with the head in a more eccentric position. Bárány (25) found that he could alleviate the unpleasant sensations experienced on a scenic railway by flexing his head 90°. More recently McDonough (147) reported that during an airborne infantry maneuver simulated casualties carried supine on litters showed no sickness whatever while a like number of men exposed to the same flying conditions in the sitting position showed an incidence of 3.8 per cent.

Quix (194) was the first to ascribe the effect of altering the position of the head to a change in the relation of otolith organs to the plane of motion. He recommended that on shipboard the susceptible individual should lie down with head inclined slightly backwards. He pointed out that in this position the utricular otoliths lie on "the blind spot of the static sense," a spot where they are lower than the ciliate cells and so do not lie on them. He believed that in this position the saccular otoliths may be influenced by rolling movements but "the rolling angle is so small that its influence is of a very limited importance."

Tyler (220, 221) found that body position is an important factor. When, during transfer from ship to shore in small landing barges troops were made to crouch, the incidence was 30 per cent as compared with 11 per cent when they were allowed to stand. Since in both postures the head is in relatively the same position it was concluded that other factors were operating. Exclusion of visual orientation as a result of crouching was doubtless one factor. Furthermore, since it is necessary for the development of motion sickness that the sense organs of the labyrinth maintain for some length of time a particular pattern of afferent discharge, the relatively greater fixation of the head in the crouching position must be considered a factor favorable to the production of seasickness.

The most extensive studies of the role of head position are those of McIntyre (155), Manning and Stewart (143–144a, 217) and Howlett, Wardell and Brett (112, 113). Manning and Stewart (143), in a study of 825 men, found marked differences in the incidence of swing sickness due to variations in posture (position of the head). In the supine position with the eyes open 5 per cent of the subjects became ill, while in the sitting position with the eyes open sickness was increased to 27.5 per cent. The maximum susceptibility occurred in the sitting position with the swing covered (64%), and it was high in this position with eyes closed (51%) or covered with black-out goggles (57.5%). They attributed the postural effect to alterations of the position of the vertical canals and offered as further support for this contention

the fact that the prone position resulted in more sickness than the supine. They concluded that any head posture in which the vertical canals assume a nearly horizontal position is the least conducive to the production of swing sickness. However, as indicated presently, there is another explanation of the results. Stewart and Manning (217) also reported that on the 4-pole swing the least susceptible position is sitting with the head turned back 90° from the horizontal. McIntyre (155) obtained similar results. In a group of 25 subjects swung in the supine position with head normally inclined the incidence was 16 per cent; in 25 swung in the supine position with head raised the incidence increased to 40 per cent; a somewhat higher incidence, 52 per cent, occurred in the prone position with head extended. McIntyre, however, concluded on morphological grounds that "the sensory end organ mainly responsible for motion sickness appears to be the utricular otolith organ of the labyrinth."

In a carefully controlled series of experiments involving 790 tests on 320 subjects, Howlett, Wardill and Brett (112, 113) investigated the effects of a variety of body and head positions on the incidence of swing sickness. With the subjects blindfolded they found that, regardless of the position of the body, the assumption of different positions of the head in relation to the radial (linear) accelerations of the swinging motion greatly affected the sickness rate. They chose as a reference for the position of the head "the plane of lines joining each external auditory meatus to the lateral canthus of the eye of the same side." Each subject was swung for 30 minutes unless sickness occurred earlier. All but 20 of the subjects were unselected air crew trainees. When 100 of these men were swung in the sitting position with the reference plane parallel to the base and perpendicular to the radius of the swing the incidence of sickness was 61 per cent. In 50 men swung also in the sitting position but with head tilted backward so that the plane was perpendicular to the base none became sick. Similarly, no sickness occurred in 50 men swung in the supine position with occiput down (plane again perpendicular to base of swing). On the other hand, the incidence was 68 per cent in a group of 50 subjects swung in the supine position with neck so flexed that the reference plane was parallel to the base of the swing. Obviously these marked differences cannot be related to the position of the body; they are clearly related to definite positions of the head. That the effect operates in highly susceptible individuals was shown by results obtained with a group of 20 men who had been eliminated from training because of persistent airsickness. In the sitting position, with reference plane parallel to the base, all became sick. Yet when they were swung in the same body position but with head back (reference plane perpendicular to base of swing) none was sick.

According to Quix (194) the base of each utricular macula lies parallel to the reference line used by Howlett, Wardill and Brett. This consideration, together with the evidence that repeated accelerations in the vertical plane, which stimulate the utricles, are effectively nauseating, led Howlett and Brett (111) to propose a theory of the mechanism of utricular response to this motion. In elaborating the ideas of Quix they suggested that the effective force due to swinging motion acts on the utricle in such a way that, depending on the position of the head, the otolith exerts a force on the macula or parallel to it or away from it. The results of the experiments cited above suggested that "stimulation for motion sickness is greatest when the force

tends to move the otolith onto the membrane, least when parallel to the membrane and intermediate when away from the membrane." Now Quix (194) had pointed out that although the base of the utricular membrane is parallel to the reference plane adopted by Howlett, Wardill and Brett, its anterior tip is curved upward. If the utricle is the sense organ that responds to accelerations in the vertical plane, it should be possible to obtain differences in sickness rates when the *g* changes occur in a plane parallel to the base of the macula but perpendicular to its upturned anterior tip. It is significant that Howlett, Wardill and Brett found an incidence of 18 per cent when the subjects were in the prone position with reference line perpendicular to the base of the swing (otoliths forced toward tip of membrane) and no sickness when they were in the supine position with the line also perpendicular (otoliths forced away from tip of membrane). Thus the hypothesis of Howlett and Brett has received substantial support. In a personal communication, however, McIntyre (156) states that, although on the swing different head positions produce different sickness rates, the results obtained on a vertical accelerator (a spring elevator) do not follow predictions based on the results of the swing experiments. He is of the opinion that the alleviating effect of certain head positions is only evident with composite motions.

The rotating-tilting machine used by Spiegel and his co-workers (216), which has been described above, imparts to the labyrinth of the subject a motion in which the velocity is rapidly and regularly altered throughout rotation of the chair at constant velocity. This exerts effects on the endolymph of the canals and so affects the cristae. The tilting also produces rhythmic vertical movements of the head, but these are of small amplitude and it is perhaps doubtful that they are of any significance in the genesis of the sickness produced. The total effect of the combined motions upon the maculae is not easily determined. The incidence of sickness was not affected by tilting the body as well as the head. Tilting the head in the frontal plane was somewhat more effective than tilting it in the sagittal plane. The highest incidence (80.8%) was obtained when during rotation a succession of head movements in the sagittal plane was followed by a succession in the frontal plane.

Although the controversy as to which one or ones of the sense organs of the non-auditory labyrinth are essential for the production of motion sickness, particularly seasickness and airsickness, remains to be settled, the results of these studies of the effect of head position afford almost a superabundance of evidence that labyrinthine stimulation is the primary factor in the etiology of motion sickness.

Central Nervous Mechanisms. On exposure to an effective type of motion sense organs of the non-auditory labyrinth discharge repetitively in some particular pattern to the brain. The maintenance of this afferent bombardment for some length of time seems essential for the development of motion sickness. The subjective experience called nausea, the visceral changes mediated through autonomic efferent pathways and, finally, the integrated somatic nervous discharges to skeletal muscle which cause retching and vomiting all result from the activation of one or more central mechanisms by what appears to be a most striking instance of temporal summation of afferent impulses. It is likely that a delimitation and physiological characterization of these central processes would add substantially to our understanding of motion sickness. When one considers *a*) that the drugs which have proved useful in the pre-

vention of motion sickness (see below) exert pronounced central effects and *b*) that there is little, if any, reason to suppose that their peripheral actions contribute to their effectiveness, it is probable that an elucidation of the central aspects of this problem may lead to a more rational therapy. A number of experimental facts bearing on the location of the essential central machinery are now available.

The role played by the cerebral cortex has been examined by two groups of workers. In studies of swing sickness in dogs Bard and his collaborators (26, 27) found that the following cortical ablations do not significantly alter the susceptibility of these animals to the emetic action of motion: bilateral temporal lobectomy; removal of both frontal poles (containing the somatic motor and sensory areas); removal of all neocortex except both frontal poles; ablation of all cortex on one side and removal of all neocortex on the other except the frontal pole. These results indicate that if any one portion of the cerebral cortex is essential for the development of motion sickness in the dog it lies outside the temporal, occipital, parietal and frontal areas of the neocortex. The question whether motion sickness depends on the functional integrity of any part of the cerebral cortex can be answered satisfactorily only by determining the effects of complete decortication in susceptible animals. In any such study it is important to make sure that the general health and nutritive condition of the animals are maintained, for there is evidence that when these are impaired a normal dog shows a lessened sensitivity to motion (26). At the time of their report Babkin and Schachter (23) had studied one dog in which the cortex of both hemispheres had been totally or nearly totally removed. Although the brain had not yet been examined, the animal behaved entirely like a completely decorticate preparation. Before operation the dog vomited, on the average, 20 minutes after the beginning of swinging. During its decorticate career it was repeatedly swung for periods of 60 minutes or more and, with one exception, never displayed any sign of motion sickness except a little salivation. On a single occasion, 2.5 months after operation, vomiting occurred after 15 minutes on the swing, but 5 days later the dog withstood the motion for 130 minutes without showing more than some salivation and chop-licking. This experiment, while most suggestive, is perhaps not entirely conclusive.

In the course of a study, partially reported recently in abstract form (27), typical motion sickness was induced in a chronically *decerebrate* dog which survived the operation for 145 days and was in excellent condition during the test periods. Before operation it was swung 7 times at weekly intervals and vomited on each occasion (times to vomiting, 4–10 minutes). The animal was decerebrated by removal of a wedge of tissue made up of caudal diencephalon and rostral mesencephalon after exposure of tectum and thalamus by bilateral ablation of occipital cortex, hippocampus and geniculate bodies. Autopsy showed that the rostral face of the brain stem remaining caudally passed from the cranial borders of the superior colliculi to a level about 1 mm. rostral to the origin of the third nerves. On each of 9 swingings carried out at weekly intervals between the 26th and 81st postoperative days the dog salivated excessively and vomited (times to vomiting, 3–9 minutes). Between the 117th and 138th days almost identical results were obtained in a second series of 4 tests.

The fact that the best known suprasegmental representation of the vestibular receptors lies in the cerebellum led Bard and his collaborators (26, 27) to examine the possibility that this part of the brain is concerned in the production of motion sickness. A dog which, in 11 swings at weekly intervals, had invariably vomited within 8 to 25 minutes and had always shown profuse salivation with licking and swallowing, was subjected to uncomplicated removal of the entire cerebellum (confirmed by post-mortem histological examination of the brain) and then studied over a period of 17 months during which time it remained in excellent health. In each of the 15 postoperative tests on the swing (14 of 60 minutes, one of 2 hours) the animal failed to vomit and the small amount of salivation and licking observed was always associated with panting. An interval of 3 months between the 12th and 13th tests and one of 6 months between the 13th and 14th swingings served to demonstrate the improbability that the immunity was due to an augmented capacity to adapt to motion. Very similar results were obtained in a series of previously susceptible dogs after removal of nodulus, uvula and pyramis. These animals, however, showed mild salivation and some licking in the absence of panting. In control experiments it was found that ablation of all parts of the vermis between primary fissure and pyramis or removal of pyramis and a few folia of uvula failed to reduce in any way the sensitivity of the animals to motion. The conclusion was drawn that nodulus and uvula contain neural mechanisms which are prepotently involved in the genesis of motion sickness in dogs. Evidence from various sources indicates that uvula, nodulus and flocculi (possibly also lingula) comprise the 'vestibular' portion of the cerebellar cortex. The results of these experiments leave uncertain the role of lingula and flocculi in the genesis of motion sickness. The slight symptoms (salivation and licking) noted above may have been due to the functional integrity of these structures.

It is interesting to note that the dog rendered immune to motion by decerebellation showed a normal sensitivity to the emetic action of apomorphine (a drug which acts directly on the bulbar vomiting 'center') and that the animals in which a partial cerebellar removal had abolished the emetic action of motion occasionally vomited in their cages after eating. These facts show that the bulbar vomiting center lies 'downstream' from the specific central mechanisms which are essential for the vomiting of motion sickness. Further, they indicate that susceptibility to motion sickness is independent of susceptibility to certain other emesis-producing agents and conditions. Bard, Woolsey and Snider (26), in a study of a series of dogs representing all degrees of susceptibility, could not find any correlation between sensitivity to swinging and the threshold emetic dose of apomorphine determined by quick intravenous injection. Birren, Stormont and Pfeiffer (37), however, in studies of human subjects, obtained some indication that the reactions to apomorphine permit a rough differentiation between susceptibles and non-susceptibles.

Another approach to the neurological aspects of motion sickness has been made through electroencephalographic studies. Neither Jasper and Morton (119) nor Lindsley and Wendt (130) were able to secure any evidence of a correlation between the characteristics or deviations of the electroencephalograms of subjects and their susceptibility to motion as determined by histories. Thorner (219) studied the electroencephalographic records of 130 consecutive cases of airsickness. Thirty-

two of these men yielded records containing unusual features. Although marked correlations were found between such recordings and independently made psychiatric estimates, it was concluded that the electroencephalographic changes could not be used as a means of pre-selecting susceptible individuals.

Physiological Status and Susceptibility. The notion that susceptibility is connected with a state of 'vagotonia' or 'sympatheticotonia' or with some instability of the central control of the autonomic nervous system is an old one to which some reference has already been made. Recent work has shown, however, that susceptibles and non-susceptibles cannot be distinguished on the basis of high or low resting blood pressures or heart rates or by changes in these on exposure to motion (31, 57, 94, 110). Wendt and his co-workers (12, 13, 90) found no differences between highly susceptible and non-susceptible individuals as regards their autonomic responses to such procedures as the cold pressor test, breath holding, hyperventilation, body tilting and injections of epinephrine or mecholyl.

Several attempts to correlate susceptibility with a greater than normal reaction to acetylcholine or to an anticholinesterase have been made. Babkin and his collaborators (21, 22) reported that in dogs there is some relation between susceptibility to small doses of acetylcholine and susceptibility to swing sickness, and they also found that when non-susceptible dogs were pre-treated with small doses of prostigmine they showed symptoms of sickness when subjected to swinging. Best, Sellers and Stephenson (32) concluded from experiments on a small series of dogs that there is probably some relationship between susceptibility to acetylcholine and to swinging. Goehring and Schwab (83) found that the injection of 1 mg. of neostigmine evoked no appreciable effect in individuals who were non-susceptible or who proved to be capable of rapid adaptation to motion, but induced all the signs of motion sickness in members of a group of men hospitalized because of chronic seasickness. On the other hand, Birren, Stormont and Pfeiffer (37), in another Navy study of quite similar material, found that the responses to the administration of this same amount of neostigmine, given by the same route, completely failed to distinguish between personnel surveyed to shore duty because of chronic seasickness and unselected representatives of the general population. Manning (140), in a study of 14 persistently airsick and 31 non-airsick trainees, did not find any statistically significant differences in blood cholinesterase activity between the two groups.

Conditions in the gastro-intestinal tract do not appear to be of significance in the determination of susceptibility, unless of course they are ones which tend to evoke nausea and vomiting in the absence of motion. Contrary to a popular conception, the interval after meals does not appear to affect the incidence of motion sickness (9, 31, 143). Schwab (201) has commented on the high percentage of gastric, pyloric and duodenal abnormalities found in patients hospitalized because of chronic seasickness. Almost certainly these disturbances were the results of long-continued bouts of seasickness and had nothing to do with the original susceptibility of the men to motion. Results obtained by McDonough (148) and by McDonough and Schneider (151) in gastro-intestinal studies of immune and susceptible subjects (see below) appear to justify the conclusion that "there is no specific type of gastro-intestinal tract, either from an anatomic or functional standpoint, which is associated with motion sickness susceptibility" (151).

Hemingway (96) found no significant difference in the incidence of swing sickness in cold and warm environments (range, 0° to 40° C.). A similar result was obtained by Magladery (136) and by Manning and Stewart (143). In a study of motion sickness in dogs, Best and his co-workers (32) could obtain no evidence that susceptibility is related to the level of ionized calcium in the blood. They also reported that a high potassium or high sodium diet does not appreciably affect sensitivity to motion.

Bodily Changes Evoked by Motion and Their Etiological Significance. A number of bodily distrubances mechanically evoked by motion have been regarded as primary or contributing causes of the trouble. For example, it has been suggested that the motions of a ship cause gross movements of heavy abdominal viscera, probably with tugging on the mesentery or traction on nerve plexuses, and that these mechanical effects are important factors in the genesis of motion sickness (121, 124, 126, 177, 189). Consequently a number of authors have tried or advocated the application of abdominal supports in the control of seasickness (43, 61, 101, 126, 189) and airsickness (233). No one has provided conclusive evidence that this measure has any beneficial effect. Aiken, Hoffman and Howlett (4) found that the wearing of a modified visceroptosis belt did not produce any significant diminution of the incidence of airsickness in a group of air navigator trainees. Although Mainland (see 165) found that the position of the heavy viscera might change as much as 5 cm. as a result of tipping the body, no correlation could be found between susceptibility and lability of the viscera. Other pertinent considerations also lead to the conclusion that visceral displacement cannot be of any real significance in the production of motion sickness. Jolting movements such as those which occur in horseback riding or during certain strenuous forms of play do not evoke nausea and vomiting, while many swaying motions of far less violence are very apt to bring on sickness (5, 138). Howlett, Wardill and Brett (113), in discussing their demonstration that the incidence of swing sickness may vary from 0 to 90 per cent with different head positions, while the position of the body in relation to the motion remains the same, have very properly pointed out that visceral displacement cannot be a significant factor in the genesis of this disorder.

Some etiological importance has been attributed to changes in the distribution of blood induced mechanically by movements of a ship. More than 130 years ago Wollaston (232), impressed by the oscillations produced in the mercury of a barometer by the up and down movements of a ship, propounded the theory that the blood in the vessels behaved in a similar fashion and so caused an alternate engorgement and anemia of the brain. Even quite recently this idea has had its adherents. Pflanz (187) made observations which led him to think that motion induces rhythmic changes in cerebral blood flow which have etiological significance. Genée (80) applied a collar to improve the cerebral circulation and reported some seemingly beneficial results of the procedure. Nunn (177) suggested that engorgement of the splanchnic vascular bed occurs and may be productive of motion sickness. Biehl (33) declared that a very important factor in the development of seasickness is the induction of mechanical changes in those parts of the brain which appear to float in cisternal fluid. None of these hypotheses has been supported by any factual evidence. As regards the supposed mechanical effect of motion on reducing cerebral blood flow, it may be noted that Spiegel, Henny and Wycis (215) found that the slight reduction in flow during rota-

tion is chiefly brought about indirectly through reflex vasomotor changes in the systemic circulation as a result of labyrinthine stimulation. Fraser (77) found that mild anoxia, equivalent to that obtained by ascending to an altitude of 10,000 feet, did not significantly augment the incidence of swing sickness.

In many discussions of motion sickness attention has been directed to the visceral effects which can be provoked reflexly by labyrinthine stimulation. For a more complete account of the reflex effects of labyrinthine stimulation on autonomically innervated effectors reference is made to the review by Spiegel (212). Spiegel and Démétriades (213) showed that in rabbits caloric, galvanic or kinetic (rotatory) stimulation of the labyrinth causes a reflex fall in arterial pressure, that this depends on the integrity of the sympathetic outflow, chiefly the splanchnic nerves, and that the effect is mediated centrally at the bulbar level. This reaction can be evoked from the utricular maculae as well as from the cristae ampullares (129). Spiegel and Démétriades (214) found that caloric vestibular stimulation increases the pendular movements and tonus of the small intestine and that this is a reflex mediated by vagal efferents.

Turning to the more relevant subject of bodily changes found in actual studies of motion sickness, one encounters a considerable number of observations on visceral alterations. Among these are several studies of gastro-intestinal functions. In an investigation of the effect of swinging on the gastric motility of 3 dogs, Babkin and Bornstein (20) found that the motion lowered the tonus and arrested the movements (hunger contractions) of the fasting stomach in a relatively non-susceptible animal as well as in the 2 very sensitive ones. The 2 susceptible dogs were later labyrinthectomized. After recovery from the immediate effects of the operation prolonged swinging not only failed to evoke salivation and vomiting, but also exerted no effect on the motility or tonus of the empty stomach.

The depressing effects of motion on the gastric musculature have also been observed in studies made on man. The reports of McDonough (148) and of McDonough and Schneider (151) are of special interest because of the bearing of their results on the question of a possible etiological relationship of gastro-intestinal changes to motion sickness. In roentgenographic studies of the effect of motion (swing tests) on 100 healthy young males it was found that the susceptible or 'sick' group showed a higher incidence of reduced gastric tone or reduced gastric peristalsis or both than the 'immune' group. The differences were statistically highly significant. Pylorospasm was found in some members of each group, either before or after exposure to motion, but its occurrence bore no relation to the development of sickness. There were no significant differences between the two groups as regards the effect of motion on the intestinal progress of a barium meal. Although the frequency of occurrence of a decrease in gastric tone and peristalsis was five times as great in the subjects who became sick as in those who proved to be 'immune,' only one-half of the former showed these changes. McDonough and Schneider point out that their observations did not permit them to predict which individuals would become sick on the swing, and they report their failure to find evidence of disorders or variations from normal in the gastro-intestinal tracts of cadets and crew members who were being eliminated because of repeated and disabling airsickness. These carefully

controlled studies give no support to the view that alterations in the stomach and upper intestine have any significant influence on the genesis of motion sickness.

In a study of the relation of gastric function to nausea in man S. Wolf (230) concluded that this sensation occurs only during gastric relaxation and hypomotility. His report will be analyzed with some care for the reason that it seemed to provide a basis for the use of combinations of atropine and prostigmine in the prevention or treatment of motion sickness (231). He used 3 normal subjects and one with a large gastric fistula. In 5 experiments caloric vestibular stimulation produced nystagmus and vertigo, usually pallor and sweating and sometimes tachycardia. Vomiting never occurred and nausea was induced in only one test. In the 3 tests in which gastric motility was determined, the caloric stimulus was quickly followed by an abolition or marked decrease of stomach contractions. The subject with a gastric fistula was twice exposed to the motion of a swing. In one of these tests no symptoms of sickness developed, but the vigorous gastric contractions decreased sharply after 3 minutes and were not resumed until after cessation of swinging. In the other test the contractions disappeared almost at once, but nearly 20 minutes of gastric quiescence elapsed before the subject experienced some slight nausea. This result contrasts with that obtained in the sole experiment (apparently on a normal individual) in which caloric stimulation produced nausea; the sensation began with and lasted throughout the period of gastric relaxation. In 6 experiments on the subjects who had been given a combination of atropine and prostigmine caloric stimulation did not appreciably affect the gastric contractions and failed to produce nausea, although vertigo was experienced and nystagmus occurred. In view of the fact that caloric stimulation failed to evoke nausea in 4 of 5 tests without these drugs, the conclusion that "gastric relaxation and hypomotility are essential to the occurrence of nausea" seems open to question. A pertinent consideration, which was apparently overlooked in the formulation of this conclusion, is the possibility that the atropine may have prevented nausea by its central action at the same time that the prostigmine maintained gastric contractions by its well-known peripheral action. In their swing tests, McDonough and Schneider (151), encountered several cases of nausea with increased gastric tone and peristalsis.

While it seems well established that various forms of vestibular stimulation, including motion, tend to reduce or abolish the tonus and motility of the stomach, the available experimental evidence does not justify the conclusion that these gastric alterations are in any way causative of the nausea and emesis brought on by exposure to motion.

Studies of circulatory changes induced by motion have been limited to determinations of changes in pulse rate and in systolic and diastolic pressures. The alterations found have been quite small and somewhat inconsistent (31, 47, 57, 94, 138). Cipriani and Morton (57), who followed heart rates by electrocardiographic recordings in 23 men during exposure to the motion of a machine found no constant change; of the 7 who vomited 2 showed slight decreases and 5 showed increases. Hemingway (94), in a study of cardiovascular changes in 489 normal young adults exposed to a swing test, determined that there is a definite tendency for the pulse to slow by 4 or 5 beats a minute in individuals who do not become sick or who develop only mild

symptoms when exposed to swinging. In the severely sick group a small increase in pulse rate was encountered as frequently as a small decrease. Best *et al.* (31) found that swinging tended to decrease pulse rates regardless of the development of cold sweating or pallor. Similarly, systolic blood pressures tended to fall in those who did not become sick or showed only mild symptoms, but rose slightly when vomiting occurred. It may be concluded, especially on the basis of Hemingway's comprehensive survey (94), that susceptibility to motion sickness cannot be related to any specific cardiovascular response to effective motion. The evidence that susceptibility is not associated with a high or low resting blood pressure or pulse rate has been given above.

The facial pallor of motion sickness is a striking and universally recognized characteristic of the disorder. Its onset is a useful sign of approaching sickness, and its persistence after exposure may be used as an objective indication of a slow rate of recovery which, according to McDonough (see 93), is a criterion of susceptibility.

Cold sweating, which is distinguished from thermal sweating by the fact that it has nothing to do with the normal control of body temperature, has been shown by Hemingway (92, 93) to be the most reliable indication of the onset of motion sickness. It occurs when mouth and forehead temperatures stand unchanged or are falling, and it may be profuse even in a cold environment. Hemingway points out that, since in his experiments there was little or no fear, anxiety or apprehension during the onset of sickness, the cold sweating did not depend on emotional or painful stimuli which, as is well known, readily evoke the response.

Several investigators (191, 205, 211) have noted in dogs and cats an increase in frequency and amplitude of respiratory movements when the animals were exposed to rocking, pitching or vertical rectilinear movements. In human subjects hyperventilation sometimes occurs in response to motion; it has been observed during swinging tests and in training flights. Apparently it is seen chiefly in susceptible individuals (198). It may lead to an alkalosis due to carbon dioxide deficit. In extreme cases carpo-pedal spasm and other signs of tetany may develop. This change is to be distinguished from the alkalosis and ketosis which Marrack (145) found in persistent seasickness and which are the results of long-continued vomiting and reduction in food intake. According to Meakins, Morton and McEachern (161) motion sufficient in kind and duration to produce sickness in dogs causes no significant changes in the CO_2 content, O_2 content, O_2 capacity or O_2 saturation of arterial blood. Cipriani and Morton (57) in a study of 22 human subjects found in most cases that motion produced a slight decrease in respiratory rate and a tendency to sigh and yawn. One subject responded by a rise in rate and developed tetany as a result of the hyperventilation.

Some exploration has been made of the question whether motion produces any chemical changes in the blood. Best, Sellers and Stephenson (32) found a slight decrease in plasma potassium, but no change in the acid-base balance or in the blood acetylcholine esterase in dogs subjected to motion which produced sickness. In similar experiments Babkin and his co-workers (21, 22) were unable to determine any significant or consistent increase in the acetylcholine content of blood. Fields, Meakins and McEachern (68) determined the level of blood sugar, calcium, phos-

phorus, sodium and potassium before and after motion on 13 subjects. Three vomited, 5 showed symptoms without vomiting and 5 were unaffected. The only significant changes (in 11 out of 13 subjects) were: rises in blood sugar of from 2 to 52 mg. per cent, which occurred whether or not the subject became ill; and reductions in blood phosphorus. No other significant changes occurred in the other blood bases. The reasons for the changes in the sugar and phosphorus levels are not clear. It is possible that they resulted from a release of epinephrine, due either to motion or to apprehension. It has been reported that the administration of 1 mg. of adrenalin delays the onset of symptoms in susceptible individuals (234) and that small amounts of pilocarpine or insulin (22 units) given to 5 previously immune subjects resulted in their becoming swing sick (235). It appears that any blood chemical changes induced by effective motion are small and probably wholly secondary to other bodily changes.

Changes in cerebrospinal fluid pressure amounting to 70 to 85 mm. of water have been recorded in a human subject during exposure to the up and down motion of an express elevator (165). There appears to be at hand no evidence which bears on the question whether such rhythmic alterations play any role in the genesis of motion sickness.

The vomiting induced by motion seems to be entirely like that evoked by other stimuli and conditions which bring on emesis. It is the result of activation, through central mechanisms discussed above, of the bulbar vomiting center which is responsible for the orderly combination and sequence of the various components of the act. This mechanism in the medulla discharges impulses over somatic nerves to diaphragm and abdominal muscles the contraction of which supplies the motive power for the emptying of the stomach. At the same time it inhibits respiration. These and other changes in skeletal muscle are preceded and accompanied by alterations in the autonomic innervation of the stomach and intestines which result in decreased tone and motility of the former and generalized contraction of the duodenum (93). Thus, in the act of vomiting, vigorous contractions of skeletal muscle empty the contents of a passive visceral bag. Emesis is a 'somatic,' not a 'visceral' act; the autonomically determined visceral changes accompanying it are in no way essential.

The various bodily changes which characterize motion sickness, the pallor, the cold sweating, the salivation, the gastro-intestinal alterations and the vomiting can scarcely be regarded as adaptive responses to the condition which evokes them. Indeed, from a teleological point of view motion sickness makes no sense. One cannot discern any biological usefulness in these responses to motion; they certainly are not protective. Hemingway (92) has suggested that an explanation is to be sought in evolutionary development. Perhaps it is not too naive to suggest that in the evolution of certain forms the capacity to respond to motion by becoming sick simply developed fortuitiously as a by-product of increasing complexity of organization and that natural selection had no opportunity to operate. It is reasonable to suppose that at least man and the dog, probably the most susceptible of animals, were rarely exposed to the adequate stimulus until the former invented or secured various forms of transportation and certain means of amusement.

Psychological Factors. The importance of psychic factors in the causation and

control of motion sickness is stressed in a large fraction of the papers which make up the literature of this subject. Many laymen support this etiological concept with the notion that the disorder is indicative of some psychological weakness. Psychiatrically inclined writers have attempted to support it by reference to one or more of the following: *a*) findings of emotional instability and neurotic traits in very susceptible individuals; *b*) claims that apprehension or fear cause or precipitate symptoms of motion sickness; *c*) the precipitation of seasickness or airsickness by disagreeable sights or odors; *d*) the fact that individuals may, through a process of conditioning (in the sense of Pavlov), become sick on motionless vessels or airplanes and, in a few cases, even by looking at or thinking about a moving vehicle or a rough sea; *e*) a supposed correlation between mental or physical activity and a diminution in susceptibility; *f*) stories of sailors and air gunners incapacitated by motion sickness who apparently recovered or at least responded effectively to an urgent call to action; and *g*) reports of the efficacy of non-specific agents such as a lactose placebo in preventing motion sickness.

The view that susceptibility is related to emotional instability and the possession of neurotic traits is based on the psychiatric examination of a few small groups of very susceptible individuals. Schwab (201, 202) examined 115 naval personnel who suffered from chronic seasickness. He divided them into two types. The individuals of Type I were 'constitutionally sick,' presented histories of sickness on all kinds of moving vehicles and devices, and at sea became sick at once and stayed sick so that they were practically useless members of the crew. Type II was composed of men with no history of motion sickness, who at sea carried on with a fair degree of efficiency despite severe sickness. By clinical examination Schwab found the incidence of neurotic traits and neurosis to be much higher in the first than in the second group. He reported that "74 per cent of the Type I individuals show neurotic trends of one sort or another, such as easily upset stomach, a tendency to nausea and vomiting from unpleasant sights, a labile vegetative system so that syncope is common, a history of odd dizzy attacks and so forth." According to Schwab these are vulnerable individuals who develop a definite psychoneurosis as a result of their unpleasant experiences at sea. A somewhat similar conclusion was reached by McDonough and Bond (150). In discussing the management of the airsick aviator they emphasized the fact that symptoms of motion sickness can develop in the absence of any motion. They divided airsick individuals into a 'benign' group, which constituted 90 per cent of the cases, and a 'malignant' group. In the former, sickness began late in flight, occurred only in rough weather and the symptoms were relatively mild. In the latter group sickness occurred even in anticipation of flight and severe symptoms tended to continue after landing. One-half of the 'malignant' group were said to possess such 'neurotic traits' as fear of flying, a history of great fear of heights in childhood, abnormal responses to the sight of blood, inadequate adjustments to family difficulties, and functional gastro-intestinal disorders. Similarly Bond (40), in a study of a group of navigation cadets in the process of elimination for severe airsickness, found a high incidence of "emotional predisposition to neurotic breakdown."

In considering these contentions that there is a specific causal relationship between the psychological make-up of the individual and susceptibility to motion

sickness it is necessary to bear in mind that what has been factually established is that among that small fraction (approximately 10%) of the general population which is highly susceptible some neurotic individuals can be found. A relevant question, which has not been answered, is whether neurotic trends and emotional instability are absent or occur less frequently in those who belong at the other end of the spectrum of susceptibility. Also, there is available no information as to the level of susceptibility among the distinctly neurotic members of the general population. Bond (40) has pointed out and illustrated the fact that the "mere presence of neurotic trends or grossly disturbed family relations does not mean that persons with such histories will be necessarily susceptible to motion."

When this problem was attacked by objective, well controlled psychological methods the results gave little evidence that there is a close relation between common psychosomatic complaints or personality disorders and susceptibility. Birren, Fisher and Stormont (35) studying 277 naval personnel, whose susceptibility was established by several independent criteria, found that a questionnaire, which clearly revealed a significant relation between seasickness and other forms of motion sickness, yielded no evidence of any correlation between susceptibility and any of the following: satisfaction or dissatisfaction with current Navy duty; incidence of headaches, constipation or indigestion; feeling 'weak' at the sight of blood; feeling ill at the sight of disgusting refuse; fainting when giving a blood sample or when receiving an inoculation; apprehension about any aspect of duty at sea. Birren and Fisher (34) found that both their questionnaire and the Cornell Selectee Index (designed to reveal defects of a neuropsychiatric or psychosomatic nature), when applied to a general Navy population or to a selected group of very susceptible men, indicated that 'no large share of the explanation of seasickness should rest on the personality structures of the individual.' In addition, they subjected a group of 48 men, who were so susceptible that they had to be given shore duty and were classified as chronically seasick, to a battery of personality tests. The Bernreuther Personality Inventory indicated that these invididuals had lower neurotic tendencies than the standardization groups for this test. The Cornell Index suggested that the seasick group had more psychosomatic complaints than men acceptable, by psychiatric criteria, for military duty, but fewer than is found among psychiatric rejectees. The more subjective tests, the Murray and Rorschach, gave some indication that the group contained more deviates than might be expected in an unselected sample. Birren and Fisher expressed the view that the personality irregularities disclosed were separate entities, associated with chronic seasickness chiefly because there entered into the selection of the men studied the great probability that a commanding officer "is more prone to request the transfer of a man who is a trouble-maker in addition to being highly susceptible to seasickness than of a man suffering from chronic seasickness alone." It was concluded that the tests whose results could be compared with those found in normal populations showed that the group as a whole was "by no means distinguished by deviations in personality structure."

Some writers have expressed the opinion that fear is an important cause of motion sickness. Rubin (197) stated that "the most important causes of airsickness, as seen in a primary air forces flying training detachment, are psychogenic in origin."

While he felt that the group could not be considered emotionally unbalanced, he attributed much of their susceptibility to fear. Green (84, 85) criticized the adequacy of the idea that motion is a primary cause of airsickness and suggested that this disturbance is "a true aeroneurosis, a reaction to fear mediated through the autonomic nervous system and frequently (but not always) precipitated by abnormal motion or altitude of the airplane." Levy (125) emphasized the psychic effect of tenseness in producing airsickness. Witwer (228) agreed with the claims of instructors and the testimony of a certain number of cadets that most airsickness in training is due to fear or nervousness. Zwerling (237) reports that electric shocks applied during rotation in a Spiegel chair increases the incidence of motion sickness. This convinces him that fear, anxiety and personality traits play important roles. Winfield (227) has characterized the recurrence of airsickness under certain conditions as "a psychological escape mechanism." Poppen (189) has expressed the opinion that the cause of air sickness is "practically always an unsatisfactory rationalization of fear," and that in seasickness apprehension is often a contributory factor. On the other hand, one finds references to occasions when the development of situations charged with danger appeared to ameliorate or cure the nausea and vomiting of motion sickness.

The weakness of the observations which have led to the claim that fear, apprehension and tension are important etiological factors is that no account was taken of the influence of visual fixation and head position or of the fact that adaptation to motion occurs under circumstances in which repeated exposures are not attended by any fear, apprehension or tension. Further, those who emphasize the importance of fear and minimize the influence of motion are silent in regard to the fact that the incidence of nausea and vomiting is so very much greater among those who become frightened on boats and planes than among those who experience fear, terror and even panic on solid ground. In his study of the relationship of airsickness to other types of motion sickness Hemingway (95) found that of 344 individuals, who had flown more than 30 hours without airsickness, 11.3 per cent vomited and 16.3 per cent showed pallor, nausea or sweating during a swing test lasting not longer than 20 minutes. It is reasonable to assume that these men experienced more tension, apprehension or fear while flying than during the swinging. Howlett and Brett (110) found that every member of a group of persistently airsick subjects showed symptoms of sickness when subjected to a swing test. They point out that "Amusement was the emotion commonly exhibited before the tests. Apprehension was at no time obvious."

It is often stated that the sight of the vomiting of another individual or exposure to a disagreeable odor induces seasickness. It is true that even ashore such experiences will cause uneasiness and even nausea in a portion of the population. Aboard a moving ship a greater number appear to be sensitive to these influences. While this may be termed a 'psychic' effect, it cannot be regarded as of primary importance in the production of motion sickness. Many of the passengers on a moving vessel are affected to various extents by the motion, that is, they suffer from various degrees of motion sickness, and it seems clear enough that certain sights and odors can serve as stimuli which facilitate the effects of the motion. Similarly, movements which alone do not suffice to produce nausea and vomiting in a given individual may bring on severe sickness if experienced during a time when some other factor is exerting a mildly nauseating effect.

It is true that highly susceptible individuals may become conditioned, especially if their work subjects them to frequent exposures to motion. Thus they may come to show symptoms of motion sickness in the absence of motion. There is also some evidence that similar conditioned responses can be established in experimental animals (26, 191).[3] In our experience, the number of individuals in the general population who do become conditioned is quite small. Adaptation is more frequently encountered. In this connection it may be suggested that the readiness with which a highly susceptible person may develop conditioned responses to odors and sights does not necessarily constitute evidence of a neurotic predisposition.

Physical activity has been recommended by many ship's doctors as a means of controlling seasickness. That any beneficial results obtained by this procedure are due to psychological effects is questionable in view of the fact that moving about may bring into operation one or more specific influences, such as positional or visual factors, which are known to reduce sickness rates. It is generally agreed among sufferers that exposure to fresh air, obtained by going on deck, relieves seasickness. That this is not due to cold air is suggested by Hemingway's demonstration (96) that there is no significant difference in the incidence of swing sickness in cold and hot environments. Again, the contention that it is attributable to some psychological change fails to take into account the fact that going on deck is likely to bring into action ameliorating visual factors. Mental activity has been regarded as an effective means of diverting attention from the malady and so of gaining relief, but the mental activities of aerial navigators and radio operators do not protect them from motion sickness, for these individuals show the highest sickness rates found among members of an air crew (84, 91, 93).

An argument which has very often been advanced in support of the view that psychological factors play a prominent part in the genesis of motion sickness is that a placebo will reduce the incidence. On *a priori* grounds this seems reasonable in the case of individuals familiar with the nauseating and emetic effects of motion. On the other hand, as Smith (206) has pointed out, the administration of placebos to a group of men naive in respect to the potentialities of exposure to motion should, on the same assumption, augment the sickness rate by making the subjects aware of the possibility of becoming sick. Since the impression that a placebo is protective is widespread and has greatly influenced thinking on the subject of the etiology of motion sickness, we shall examine in some detail the available evidence pertinent to this question.

A number of reports of sickness rates in tests on experimental devices have referred to placebo effects. In their early study of swing sickness, Brown, McArdle and Magladery (47) found in a group of subjects (number not given) that "Dummy tablets (lactose) produced no significant difference in the results of swinging except

[3] It should be emphasized that the development of conditioned vomiting as the result of repeated exposure to effective motion is rare among dogs. Although Bard and his collaborators, in an extensive series of observations (26, 27) on susceptible dogs subjected repeatedly to the motion of a swing, encountered a few instances of salivation when the animals were brought into the experimental room or placed on the motionless swing, they never saw conditioned emesis. Furthermore, Noble (see footnote 1) reports that "When animals were swung every 7 or 5 days even though this was continued over a period of years, no case of conditioning was ever encountered." In dogs, as in men, repeated exposure to an effective motion is more apt to result in a decrease than in an increase in susceptibility.

in one individual." Fields, Campbell and Penfield (66) in drug trials on naval ratings, who without any treatment had experienced nausea or had vomited during an initial test on a swing or rocker, gave placebos to 14 men. They state: "Only two (14%) were protected by the treatment and one (7%) partially protected. All three of these had been completely protected by V-1 about a week previously suggesting either a prolonged effect of V-1 or a psychological effect from having been previously protected." The small number of subjects used hardly permits any conclusion regarding protection. In Noble's trials of the effect of drugs on swing sickness (173) there was only one experiment which could possibly yield information about the effect of a placebo. One week after their first test, for which they had been given a capsule of lactose and in which they went the full 30 minutes without vomiting, 24 subjects were swung without any treatment. Three vomited. One of these belonged to a subgroup of 11 who on the first trial had received lactose in pink capsules "at a time when the protective action of pink capsules against seasickness was widely publicized." The other two were among the subgroup of 13 which had been given the sugar in ordinary white capsules. Noble's comment on the result of the second test is: "This would suggest that 12 per cent may have been protected by placebo treatment. On the other hand it may represent the number of individuals whose time of vomiting varies from 25 to 35 mins." Also he concludes that placing the lactose in pink instead of white capsules did not enhance its value as a preventive (see also 183).

In swing trials of various proposed remedies several groups of Canadian investigators (64, 184, 186, 203) used more than 2000 subjects who were graded as susceptible on the basis of the development of severe symptoms during a test which followed the administration of a placebo. Subjects who appeared to be protected on a second placebo trial were removed from the results of the therapeutic trials. Sellers, Parker and Stephenson (203) rejected 67 (28.6%) of 234 subjects because they were either "improved by the psychic effect of the capsule" or "varied from their state at the initial swinging." In a total of 51 tests Parker, Sellers and Stephenson (186) found that the average time on the swing was 29.4 minutes (20 trials) when the placebo trial followed the therapeutic trial and 26.0 minutes (31 trials) when it preceded the testing of the remedy. On the fifth test Fields (64) administered placebos to 46 subjects who had become sick on their first test which was also a placebo trial. The results indicated that 8 were protected and 6 improved (a total of 30.3%). But since the fifth test had followed the trial of an effective remedy by only 3 to 5 days, Fields questioned the validity of ascribing the protection to the placebo. Most of these reports, however, place some emphasis on the supposed protective action of a placebo, but none unqualifiedly asserts that such protection is the only explanation of the results obtained. Doubtless re-testing with a placebo is a necessary check on the susceptibility of the subject during the course of such therapeutic trials. It may serve as a rough correction of the percentage protection observed during the previous trial. But under the conditions of these experiments failure to become sick during the second test of a placebo cannot be taken as evidence of a psychological effect. Indeed the writers of these reports acknowledge the possible operation of two other influences: a prolonged action of a drug given a few days previously, and variations in a given individual's time of vomiting. A third influence is of course the adaptation which

occurs in the majority of susceptible subjects when they are exposed repetitively to the motion of a swing (120, 141), even when successive tests are at weekly intervals (118).

In their recent review of the therapeutic studies sponsored during the War by the Canadian National Research Council, Noble, Sellers and Best (175) state: "In all sea trials definite protection (of the order of 20 to 30%) was afforded by placebo treatment as compared with untreated groups."[4] This declaration is apparently based on results summarized in a subsequent paragraph which refers to tests carried out on troop transports (large passenger liners). These results, originally reported by Parker *et al.* (185), were as follows: On the first day of *trip 1* a '1st sitting' of 220 men received placebos and showed a sickness rate of 10.5 per cent, while only 4.1 per cent of the members of a numerically equal '2nd sitting,' who were given a remedy, became sick. On the second day, with sea conditions the same, neither group received anything and the sickness rates were 17.3 and 15.5 per cent, respectively. Even if it were justifiable to compare the sickness rates (10.5 and 17.3 per cent) of the '1st sitting' on successive days, it is evident that for the numbers used the difference is without statistical significance. In the case of *trip 2*, at the outset of heavy weather on the fourth day, 200 subjects were given a remedy, 160 a placebo and 211 nothing. The sickness rates (incidence of nausea or vomiting) in these three groups were 6.0, 12.5 and 28.4 per cent, respectively. Although the difference in incidence between the placebo and untreated groups is statistically significant, the absence of any information about the method of selection of the groups and their distribution, supervision and duties aboard the large ship raises some doubt that the placebo actually exerted an effect. In another trial aboard a transport the sicknes rate was 36.4 per cent in a group of 85 men who were given a placebo and 51.7 per cent in a control group of 87 who received nothing (181). Parker, who reported this work, did not claim that the difference indicates a placebo effect and he was careful to point out the unsatisfactory conditions of the experiment. The subjects were divided into groups not at random but on the basis of their boat stations during emergency drill; the men belonging to the placebo and control groups were not from comparable parts of the ship; there was no supervision of them during the experimental period; and, because they were French Canadians, the symptoms experienced were determined by group questioning through an interpreter.

To the best of our knowledge the facts set forth in the last three paragraphs constitute the entire material on which claims of the efficacy of a placebo may be made. Standing in contradiction to this material are the results of three investigators who examined the problem under more favorable and better controlled conditions. In his study of the effect of hyoscine on airsickness Lilienthal (128) was able to compare the sickness rates which followed the giving of a lactose placebo with those obtained when nothing was given. His subjects were cadet navigators homogeneous as regards age, daily activities and flying experience. Previous to the study they had had 200 or more hours in the air and thus had probably undergone considerable adaptation. The only uncontrolled variable in the flights was the degree of turbulence

[4] The reference given after this statement must be a typographical error, for it is to the analysis by Luykx (132) of the results of sea trials in which there were no untreated groups.

"but the study was extended sufficiently long to make the individual test groups comparable." The control procedure was 531 cadet-flights without treatment during the first two months of observation; it resulted in a sickness rate of 7.5 per cent. This was followed by a period in which, on 200 cadet-flights the incidence fell to 0.5 per cent as a result of giving hyoscine. During the last period, when a placebo indistinguishable from the hyoscine was given, the rate was 6.3 per cent in 239 cadet-flights. The reduction in the sickness rate from 7.5 to 0.5 per cent is impressive, the possibility that it was due solely to chance being less than one in 1000. The difference between 7.5 and 6.3 per cent is without any statistical significance. Smith (207) also was unable to find any effect of a placebo on the incidence of airsickness. His subjects were 274 navigation students selected in the sense that most of them had been airsick 3 or more times when they were given, before each of 5 successive flights, one of the following: remedy A, remedy B, remedy C, a placebo or nothing. The order of these procedures was changed in successive flights and there were 961 individual tests. A placebo was given in 204 tests, nothing in 187. The incidence of vomiting was 41.2 per cent in the former, 42.3 per cent in the latter. On the other hand, actual medication resulted in vomiting rates of 13.5, 19.9 and 21.5 per cent for the 3 remedies tested. In Tyler's 4 experiments (220, 221) involving 563 unselected young men (infantrymen) undergoing shore-to-shore amphibious training in small landing craft (LCVP's) the incidence of seasickness averaged 35 per cent (range, 30–43%) in the 4 untreated groups and 34 per cent (range, 26–46%) in the 4 groups that had been given a placebo and *told it was an effective remedy.* Further, the incidence of severe sickness was 15 per cent (range, 8–29%) in the placebo groups and 13 per cent (range, 9–21%) in the untreated groups. In each of the 4 experiments the members of these groups, together with those of 2 other groups who had received a remedy, were distributed at ramdon in the several identical boats and exposed simultaneously to the same sea conditions. Further, none of the observers and recorders knew the distribution of the men belonging to the 4 categories. The results of these 3 well controlled studies, each differing from the others in the kind of subjects used, make most dubious any assertion that the giving of a placebo with its attendant suggestion can influence the incidence of motion sickness in groups of normal young individuals.

Relevant to a consideration of possible psychological factors in the etiology of motion sickness is the fact, emphasized by Smith (206), that many drugs which possess sedative, hypnotic or anesthetic properties are quite ineffective in preventing motion sickness. While several barbiturates have been shown to depress the emetic response to motion this characteristic is not necessarily related to their hypnotic or anesthetic properties; actually the most effective of these has convulsant rather than hypnotic properties (173). An observation which possibly bears on this question is that in the dog removal of the entire cerebrum in no way diminishes susceptibility to motion sickness, whereas ablation of a small subdivision of the cerebellum abolishes susceptibility (see above).

In summary, it can be stated that the results of relevant, adequately controlled studies do not permit the conclusion that psychological or psychopathological factors are of importance in the etiology of motion sickness. Finally, it may be pointed out that such factors may exert an influence after sickness has developed, for it is well

known that they affect the behavior and the capacities of individuals during the course of any disability.

SELECTION TESTS AND PREDICTION OF SUSCEPTIBILITY

During the War a good deal of work was devoted to attempts to develop methods which might serve to predict susceptibility to motion sickness. It became obvious that much time, effort and expense would be saved if it were possible with accurary to exclude from certain training programs, especially those of the air forces, individuals of high susceptibility. Three types of procedures were investigated: special vestibular function tests, tests on swings and other effective experimental devices, and the taking of individual histories of response to motion by means of questionnaires.

Vestibular Function Tests. Early students of this problem attempted to associate susceptibility to motion sickness with abnormal responses to the Bárány chair test. Because nausea and vomiting occasionally occur in rotation tests and because of the evidence that motion sickness is vestibular in origin, the United States Army Air Forces for some time required that all candidates for flying training, who had a history of motion sickness, take a specified Bárány chair test (49, 93). Investigators at the AAF School of Aviation Medicine, Randolph Field, studied the question whether this test could be used as a means of prediction. Campbell and his collaborators (53) applied it to 58 subjects, 30 of whom had definite histories of immunity or susceptibility. They failed to find a good correlation between abnormal responses to rotation and susceptibility, but because of the smallness of the group definite conclusions could not be drawn. McDonough and Thorner (152), in a more extensive study involving over 400 men, were unable to detect any difference between the incidence of swing sickness or airsickness in two groups, one of which qualified and the other failed to qualify in the Bárány chair test. Accordingly the test was abandoned for the purpose of predicting airsickness (97).

Spiegel and his collaborators (216) suggested that their rotating-tilting machine might be used as a means of selection. They found that the weaker method of stimulation (rotation combined with sagittal movements of the head) would reveal highly susceptible individuals.

Caloric tests of vestibular function have also been employed in attempts to predetermine susceptibility. Goehring and Schwab (83), who tested by irrigating one external auditory canal with 25 cc. of ice water, reported that "subjects who were susceptible showed persistent and marked vertigo, nystagmus, nausea and a degree of falling reaction. Non-susceptible men had no such symptoms nor any transient vertigo or nystagmus." On the other hand, Morton, McNally and Stuart (165, 167) could not find any definite correlation between the vestibular responses of 25 subjects to minimum cold caloric stimulation and their liability to sickness on an effective motion machine. At least two groups of English investigators (47, 86) have reported that there is little correlation between the type of nystagmus obtained in carefully standardized caloric tests and susceptibility to airsickness. The Randolph Field group (152) found that when an evaluation scoring system was used for the responses to both the chair and the caloric tests a 'moderate degree' of correlation was obtained.

Tests on Experimental Mechanical Devices. When standard procedures were adopted for swing tests and such important variables as the position of the head, angle and frequency of swinging, vision, etc. were controlled, the results of most investigators have indicated a high degree of correlation between susceptibility to swing sickness and to airsickness. Hemingway (95, 97) obtained the following sickness rates in uniform swing tests: in 438 non-selected, pre-training glider pilot candidates, 28.6 per cent; in 16 cadets hospitalized for airsickness, 100 per cent; in 35 airborne infantrymen who were airsick in a maneuver, 80 per cent; in a group of 73 navigators who were about to be eliminated because of airsickness, 91 per cent. Park (179) found a 67 per cent correlation between swing sickness and airsickness. Brown, McArdle and Magladery (47), using a 15-minute bout of swinging supplemented by 7 minutes of rotatory movements, found that of 15 individuals who had been rated as susceptible to airsickness 10 became sick, while only 5 of 22 inexperienced subjects succumbed. Holling and Trotter (104, 106), however, reported that they were unable to find a good correlation between susceptibility to swing sickness and a record of seasickness. Noble (173) is of the opinion that swing tests are too severe and tend to classify too many as susceptible.

Canadian investigators (63, 165) reported a good correlation between a past history of susceptibility to sickness on ships, aircraft or trains and results obtained on the 'roll-pitch rocker.' Of 26 'immune' subjects only 1 (4%) showed severe symptoms during 30 minutes on the machine while 28 of 41 'susceptibles' (68%) became sick. In 108 men with unknown histories the incidence on the machine was 32 per cent.

In their study of 477 naval officer-candidates Wendt and his co-workers (10) analyzed the relationship of the results obtained on the vertical accelerator to previous history of motion sickness as determined by a questionnaire. The results of this carefully controlled study "showed a reliable and moderately high relation between sickness history and experimentally produced sickness." The over-all rates of sickness on the accelerator were: 45 per cent in susceptibles, 24 per cent in intermediates, and 14 per cent in the group of non-susceptibles. The corresponding 'sickness indices,' determined by giving a double weight to vomiting and single weight to lesser symptoms, were 65, 35 and 21. It was concluded that the sickness evoked by the machine "has factors in common with other forms of motion sickness."

Despite the good correlation reported by a number of workers, it appears that both unnecessary elimination and undesirable retention would occur if selection were based on performance in a swing test. McDonough (149) found that 65.7 per cent of 380 navigator cadets were airsick on one or more occasions; the average was 3 times. Five per cent were eliminated because of airsickness, while the remaining 60.7 per cent were graduated in spite of the fact that they had been airsick on occasion. If susceptibility to swing sickness had been used as a means of prediction 14 of the 19 who were eliminated would have been dropped from the training program, but 130 others of whom 39 never became airsick would have also been eliminated with them. Park (179) found that on the basis of a swing test lasting 30 minutes 12 per cent of a group of candidates would have been rejected unnecessarily and 19 per cent would have been accepted for training and subsequently found unsuitable because of susceptibility to motion sickness. When he reduced the time of swinging to 20 minutes,

the results showed that 7.5 per cent would have been rejected unnecessarily and 20 per cent, later found to be unsuitable, would have been accepted. A further reduction in swinging time to 15 minutes gave essentially the same result. Joekes (120) has suggested that the use of a single swing test is of little help in pre-selection of personnel, since it supplies no information regarding the ability to adapt. He found that of the number who were sick on the first swing test only 1.7 per cent failed completely to adapt and a further 1 per cent showed only slight adaptation. This work and that of Manning (141) strongly suggest that failure to adapt to the motion of a swing might be a reliable criterion for rejection of candidates for duties in which they are exposed to effective motions. Hemingway (93, 97) has recommended that the determination of the ability of the individual to adapt is the most practical selective procedure.

Use of Case Histories (Questionnaires). A number of investigators have examined the possibility of predicting susceptibility on the basis of a history of past experiences on various vehicles and certain amusement devices. They have used questionnaires which asked about rides on boats, ships, aircraft, trains, automobiles, buses, elevators, roller coasters, ferris wheels, merry-go-rounds, etc. One of the objections to this procedure is the question of the reliability of the answers of candidates to a questionnaire, particularly when they are aware that their military status or future may depend on their replies. The studies of Birren, Fisher and Stormont (35), of Birren and Fisher (34) and of Wendt and his collaborators (10, 225) indicate that reliance can be placed on answers obtained in properly administered inquiries of this sort and that a questionnaire may be useful as a selection or elimination device.

Hemingway (95, 97) reported a definite correlation between a history of motion sickness and susceptibility to airsickness and swing sickness. But on the basis of his study referred to in the previous section, McDonough (149) points out that if elimination of candidates were determined by histories much the same wasting of adequate personnel would occur as when reliance is put on the results of a swing test. Park (179) emphasizes the fact that if he had used a history as a basis of selection, 10 per cent of the subjects would have been rejected unnecessarily (compare with 12% by the swing test) and 19 per cent, later eliminated from training, would have been accepted.

The weight of evidence indicates a fairly high degree of correlation between susceptibility to airsickness or to the sickness produced by swings or other experimental devices and the susceptibilities revealed by a good questionnaire. Nevertheless, for efficient selection within a large group, the taking of histories, like the giving of a single swing test, probably cannot reveal the thing that it is most essential to know, namely, the ability of most of the susceptible individuals to adapt to motion.

THERAPY

Before the recent War no adequately controlled study of the efficacy of any supposed remedial or prophylactic agent or measure was carried out. In the case of seasickness the number of remedies which were advocated approximated the number of interested ship's doctors, ship's officers and highly susceptible passengers. No attempt will be made to mention all the measures that have been tried.

When in 1941 and 1942 it became evident that motion sickness would be a problem of considerable moment in war medicine serious efforts to control it began. Exten-

sive sea-borne and air-borne training operations afforded, actually for the first time, the possibility of carrying out tests on numbers of individuals sufficiently large to give results having statistical reliability. Eventually a fair number of adequate trials were performed, but those who conducted them, both in the air and at sea, were often discouraged by the inconstancy of the two elements. Such students of the problem are well able to appreciate the remark of 3 workers (105) in England: "Chronic sufferers from seasickness may be astonished to learn that on most days throughout the year an obstinate and baffling calm haunts the waters round this island." The development and wide use of effective mechanical devices for the experimental production of motion sickness in men and animals not only advanced knowledge of drug therapy, but served to bring about an appreciation of the influence of such factors as head position, vision and adaptation.

Psychotherapy. One or another form of psychotherapy has been recommended by a number of authors (30, 43, 114, 189). A few writers have reported beneficial results which they attributed to suggestion, restoration of confidence and the like, but which, if actually obtained, could have been due to the operation of other influences. For example, Levy (125), who dealt with 52 cases of airsickness among cadets during their first few hours of flying, stated that he reduced the sickness rate from about 10 per cent to approximately 2.8 per cent by instituting a 'psycho-physiological lecture,' in which the students were not only given reassuring information but instructed to throw the head back, change position and fix attention on objects on the ground when they began to feel ill. In addition, instructors were persuaded to "take over and fly straight and level" when a cadet showed the early signs of sickness. Similarly, Green (84), after concluding that airsickness is a reaction to fear, an 'aeroneurosis,' managed the problem of 35 members of a combat bomber crew training unit who had been grounded for sickness by reassuring them and giving them graduated short flights in a type of plane which was 'bumpy,' but generally regarded as 'safe.' Further, a combination of phenobarbital and atropine was administered. A a result of these measures 10 were 'cured' and a good many others showed improvement. Because they ignored such factors as the influence of vision, head position and drug therapy and failed to take into account the fact that adaptation to motion is readily obtained in the absence of apprehension or fear, the conclusion of each of these writers that 'psychotherapy' had an influence cannot be taken seriously.

The absence of any acceptable evidence that psychological or emotional factors are of substantial significance in the etiology of motion sickness (see above) suggests that psychotherapy holds little promise of aiding in the control of this disorder.

Dietary Measures. These of course have constituted a favorite subject of many seagoing physicians. Much has been written on the kind of food that should or should not be eaten by the susceptible voyager. There is very little agreement. Some recommend fasting, others only partial starvation. Special diets ranging from meals consisting of nothing more than fruit juices to repasts in which the main dish is beefsteak have had their advocates. One notable recommendation (15) was "soup made of horse-radish and rice, seasoned with red herrings and sardines"—together with small amounts of champagne. As regards the use of alcohol and tobacco, the literature is controversial. Some maintain that these should be strictly avoided; others, more

liberal, encourage the swallowing of straight brandy. While the consumption of considerable quantities of champagne is known to result in some temporary indifference to the symptoms of seasickness, experienced sailors report that disastrous results are likely to attend the practice of going to sea with a 'hangover.'

Seasickness or airsickness will occur in an untreated susceptible individual whether he have a full or an empty stomach, and the available evidence (9, 31, 143, 221) indicates that as long as his diet is not one which would provoke indisposition in a motionless individual on land it makes little difference what he eats or when he eats it.

Mechanical Measures. According to Levilly (126) and Irwin (116) Dr. Keraudren in 1812 was the first to suggest the use of abdominal binders. There have been several recent advocates of such supports (43, 54, 61, 101, 177, 189); evidently they have been impressed by the possibility that visceral displacement or shifts of blood in the abdomen are of etiological importance. In view of the fact that there is much evidence to show that such changes (even if they do occur) are of no consequence in the causation of motion sickness, it is not surprising that Aiken, Hoffman and Howlett (4) and Brown, McArdle and Magladery (47), in controlled studies, were unable to find that the use of these devices has any value. Neck cuffs have been recommended on the assumption that cerebral anemia occurs as a result of exposure to motion and is a factor in producing sickness (80), but Fraser (77) was unable to find that the incidence of swing sickness was affected by breathing low concentrations of oxygen. It has been recommended (153) that inhaling deeply when the ship rises and exhaling forcibly on the downward movement is a good prophylactic measure. Over 130 years ago Wollaston (232) advocated the reverse procedure.

At one time or another steamship lines have tried special cabins which, like a ship's compass, were swung on a pivot to prevent them from rolling or pitching. Bessemer (see 17), the inventor of the steel process, was the first to suggest this device. Passengers still became sick, for, as pointed out above, the cabins did not eliminate the vertical motions.

Many types of machines designed to adapt individuals to motion before they go aboard ship or plane have been tried or suggested. Their efficacy appears to be limited by the specificity of the process of adaptation. Gibson, Manning and Cohen (81) found that a regime of swinging for 15 minutes a day during 2 or 3 weeks before and during a period of flying failed to reduce the amount of sickness experienced by subjects in Anson aircraft. There are a number of observations to the effect that acrobats, ballet dancers and athletes are more or less immune to seasickness (43, 139, 178). There are suggestions that physical exercise involving tumbling will reduce the incidence (139). Gibson, Manning and Kilpatrick (82) presented evidence that "a simple 8-week active physical training programme including vestibular exercises" reduced susceptibility to swing sickness. Magladery (135) was unable to find that 2 weeks of physical training produced any lowering of susceptibility to sickness in gliders.

There is general agreement regarding the worth of one mechanical procedure: the assumption of a horizontal position. The reasons for the beneficial effects obtained have been set forth in the section on the effect of position.

Remedial and Preventive Medication. A review of the literature, including letters

to the editors of medical journals, from the year 1800 to the present shows that almost every item in the pharmacopeia has been recommended or tried. Some of these have been truly remarkable (14, 102, 134).

The ante bellum efforts to determine whether one or another drug or mixture of drugs is effective have been thus described, possibly too generously, by Noble, Sellers and Best (175): "In general, the investigations have been poorly controlled and are based on too few experiments. These defects make most of the findings of historical interest only." If a trial is to yield reliable results it must meet certain basic requirements. In view of the great variations in individual susceptibility, either the subjects must be ones in which this characterisic has been well established or, if unselected, the subjects must be sufficiently numerous. The reason for the latter precaution has been well stated by Holling, McArdle and Trotter (105) as follows: "If the treated and control groups were sufficiently large it would be justifiable to assume that each contained its fair share of men of every grade of susceptibility, from the immune to the most susceptible, and that the rate of sickness in the control group was therefore a good indication of what the rate of sickness would have been in the treated group if no drug had been given." This procedure is the one which must be used when it is not possible to secure the same individuals for repeated tests. Whether the controls should receive a dummy tablet or not or be subdivided into untreated and placebo groups depends on one's concern about the alleged psychological effect of giving a placebo. It is doubtless much more important to make sure that the observers cannot know which treatment (including a placebo, if used) any subject has received. For the sake of economy of numbers many of the field trials of remedies carried out during the war used only a placebo group for control; it was felt that any medication which did not significantly lower the incidence below that found among those receiving a placebo was without value. In the case of selected subjects of known susceptibility repeated tests must be managed in such a way that adaptation does not confuse the results. Another aspect of the problem of procedure which is important is the criteria of sickness used. There is now general agreement that in tests on human beings nausea and vomiting are the only readily determined symptoms which give dependable evidence of sickness. In animal experiments emesis is probably the only reliable criterion. An excellent discussion of the proper methodology for the determination of the relative value of drugs in the prevention of motion sickness, more particularly airsickness, has been given by P. K. Smith (206).

Little can be said of the curative or remedial effects of any drug, for there have been no adequate tests of the action of pharmacological agents on individuals already motion sick.[5] During the half century which preceded World War II the drugs most often employed to cure seasickness were the bromides, chloral hydrate, glycerol trinitrate, amyl nitrite, sodium nitrate, cocaine, morphine, chlorbutanol, chloroform and ether. These were prescribed individually and in various combinations. Obviously a number of them were given to induce some degree of somnolence until the trip or the bad weather was over. In view of the results of later tests of the effectiveness of belladonna alkaloids as preventives, it is interesting to note that Fisher in 1913 (71) and Bohec in 1930 (39) reported that the administration of atropine to already sick indi-

[5] But see Appendix.

viduals gave very favorable results. There were also numerous reports of the curative effect of barbiturates (87, 99, 100, 101, 158). Because of failure to carry out adequate control procedures most of these studies yielded results which gave no trustworthy information concerning the therapeutic value of the substances employed.

On the other hand, there are now available the results of a number of statistically reliable studies of the use of drugs as *preventives* of motion sickness. In its military aspects the problem is chiefly a matter of preventing the disorder during the several hours of critical amphibious and air operations. Almost all the adequate trials have been directed toward the discovery of drugs which will significantly reduce the sickness rates which may occur under these conditions. For obvious reasons the oral route of administration has been used in all such studies of pharmacological agents.

The present status of drug therapy in the prevention of motion sickness has been competently reviewed in detail by P. K. Smith for the Army Air Forces (206). Much of the material summarized below has been drawn from his presentation. Attention should also be directed to the review (175) of therapeutic studies which were sponsored by the National Research Council of Canada.

A criterion frequently used to measure the prophylactic efficacy of a drug is "the per cent protected" (206) or "susceptibles protected %" (105). It is a figure which gives an approximation of the number of susceptibles protected by the remedy and is obtained by the formula:

$$\frac{(\%\text{ sick in control group}) - (\%\text{ sick in treated group}) \times 100}{(\%\text{ sick in control group})}$$

While this figure is useful in expressing the results of a given experiment, it must be borne in mind that it is valueless if the difference in the numerator is without statistical significance. Reliable differences should have probability values (P) of 0.05 or less. Furthermore, the percentage protected may be misleading in comparing the results of different experiments, since the sickness rate in the control group, which depends on the effectiveness of the motion and the average susceptibility of the members, is a factor which determines the effectiveness of a preventive.

The pharmacological agents most extensively studied during the war were the belladonna alkaloids and central nervous system depressants such as the barbiturates. It was reasonable to place emphasis on these substances, for a considerable literature had accumulated which suggested that they may be effective. It appears that the belladonna alkaloids were first suggested for this purpose anonymously in 1869 (16) and what appears to be a possibly valid test of atropine in the prevention of seasickness was made by Beard (29) in 1880. A number of substances were tested because they appeared to relieve nausea in other disturbances such as radiation sickness and pregnancy, while others were studied for less cogent reasons. Although the urgency of the problem made it necessary to test certain drugs on purely empirical grounds, the selection of substances for final field trial was made, whenever feasible, as carefully as possible. Thus the attempt was made always to try a newly proposed remedy first on susceptible animals or on a limited number of human subjects exposed to the motion of some mechanical device. Then, if it seemed promising, a separate series of tests were conducted to determine whether or not the dosage to be used produced any undesirable side effects. In the case of compounds of unknown or poorly known

pharmacological action it was of course essential to carry out extensive toxicological studies on animals.

1. BELLADONNA ALKALOIDS. Before World War II there were available several proprietary remedies which contained these substances, but no adequate tests of their effectiveness had been carried out. Evidence which accumulated during the years 1942 to 1945 definitely established the fact that when administered 0.5 to 4.0 hours before exposure to motion, preparations containing one or more of the naturally occurring belladonna alkaloids are effective in reducing sickness rates encountered on mechanical devices (64, 66, 67, 70, 173, 175, 182, 184, 206, 210), in airplanes (127, 128, 207, 208) and at sea (18, 103, 105, 106, 132, 133, 175, 185, 220, 221).

It is of interest that these drugs, so useful in the case of man, appear to be wholly ineffective in preventing motion sickness in dogs. Babkin and Dworkin (21) gave atropine (0.25 to 0.75 mg.) to 4 susceptible dogs and then subjected them to swinging. Vomiting was neither prevented nor delayed, although salivation was abolished. Holling and Trotter (106) and Noble (172) obtained similar results on giving hyoscine to dogs. In experiments not heretofore reported Bard also found that hyoscine (0.65 to 2.0 mg.) failed to protect a number of susceptible dogs from the emetic effect of a swing. On the other hand, dogs are readily protected by a number of barbiturates and this effect is not related to the anesthetic properties of the compounds (172).

a) *Hyoscine* (L-hyoscine, scopolamine) is probably the most effective of the belladonna alkaloids. In 1942 Holling, McArdle and Trotter (103) carried out the first adequately controlled studies of drugs as preventives of seasickness. Their results, obtained on unselected soldiers subjected in groups of about 70 to the motions of minesweepers or trawlers on trips of 4 to 6 hours duration, were reported in the public literature in 1944 (105). Using as criteria of sickness either nausea or vomiting, they found that of a number of preparations tested hyoscine appeared to be the most generally useful. When 0.6 mg. of the hydrobromide was given only 20 per cent of 218 were sick whereas 46 per cent of the 212 controls (given dummy tablets) were similarly affected. This indicated that 57 per cent ($47-20/47 \times 100$) of those who would have been sick were protected. Suggestive but not conclusive evidence was obtained that 1.2 mg. is even more effective than 0.6 mg. The conclusion that hyoscine is a good prophylactic agent received limited confirmation in sea trials carried out as a joint venture by representatives of the Royal Canadian Navy, the United States Navy and the National Research Councils of Canada and the United States. The results, analyzed by Luykx (132), indicated fairly definitely that hyoscine alone (0.65 mg.) and two other compound preparations containing lesser amounts of the substance were effective in preventing seasickness under conditions of moderate roughness. Later in 1944, in the course of some 60 tests on more than 15,000 unselected troops during ship-to-shore and shore-to-shore amphibious training operations, Tyler (220, 221) showed that several drugs or combinations of drugs are effective preventives. When hyoscine hydrobromide (0.6 mg.) was given alone the ratio of the percentage of those receiving the drug (687 men) who became sick, to the percentage sick in the placebo groups, was 0.34. Here the mean incidence in the controls was moderate, namely, 20 per cent, and the average protection 66 per cent. In one trial carried out when sea conditions produced a sickness rate of 53 per cent in the control (placebo)

group of 118 men the same dose of hyoscine protected approximately 49 per cent of the susceptibles in a treated group of 125.

Hyoscine appears to be as effective, prophylactically, in airsickness as in seasickness. Lilienthal (128), whose method of study has been described above in connection with the alleged effectiveness of a placebo, found that the protection afforded by the administration of 0.6 mg. of hyoscine hydrobromide was 92 per cent on 200 cadet-flights. In his study the incidence of sickness in the control groups was quite low: 6.3 per cent in the placebo group during 239 cadet-flights; 7.5 per cent in the untreated group on 531 cadet-flights. But the medication reduced it to 0.5 per cent. Smith (207) who gave a slightly larger dose (0.75 mg.) obtained a protection rate of 67 per cent. In his well controlled experiment a placebo was given in 204 tests, hyoscine in 222; the sickness rates were, respectively, 41.2 and 13.5 per cent. In comparing the results of these two investigators it should be borne in mind that the subjects used by Lilienthal had had far greater opportunities than those employed by Smith to become adapted to motion before being given a therapeutic test. Lilienthal (128) has reported a number of instances of successful preventive therapy with hyoscine in individual subjects complaining of persistent airsickness. Haslam (89) has described similar experiences with persons whose histories indicated a very high degree of susceptibility to airsickness or seasickness.

Evidence of the effectiveness of hyoscine has been obtained in tests on swings. Smith and Hemingway (210) obtained from 44 to 59 per cent protection with doses of 0.5 to 0.75 mg. Canadian groups (173, 175, 182, 186) found that doses ranging from 0.4 to 0.8 mg. gave protection rates of from 29 to 76 per cent (175). Despite one test in which 1.2 mg. gave only 19 per cent protection (203), it is apparent that in general the size of the dose is a factor in determining the degree of protection. It is also evident that when equal doses and comparable criteria of sickness are used there is an inverse relationship between the degree of protection and the incidence of sickness in the controls.

b) *Atropine*, which is a mixture of equal parts of the optical isomers of hyoscyamine, has received less experimental attention than has hyoscine as a preventive of motion sickness, possibly because in therapeutic doses it lacks the primary depressant action of hyoscine on the central nervous system. In their sea trials Holling, McArdle and Trotter (105) found that of 88 men receiving 1 mg. of the sulfate 23 per cent were nauseated or vomited, whereas 38 per cent of 86 controls (placebo) experienced one or both of these symptoms; the protection of susceptibles was 40 per cent. Quite similar results have been reported by Smith (206) and by Smith and Hemingway (210) in studies of swing sickness, in which the dose was also 1 mg. (given one hour before exposure to motion). In a small group of subjects selected for their susceptibility the protection rate was 42 per cent. In trials carried out on 471 unselected subjects, of whom 365 were controls, this same dose gave an estimated percentage protection of 47 per cent. These relatively limited tests suggest, but do not demonstrate, that atropine is somewhat less effective than hyoscine.

c) *Hyoscyamine.* This alkaloid occurs in two forms, the D and L isomers. The usual commercial product is L-hyoscyamine. As pointed out above, atropine consists of a mixture of equal parts of these isomers. The effectiveness of L-hyoscyamine as a

preventive of motion sickness, when used alone, has been tested by several investigators, but it has been used more often in combination with other belladonna alkaloids, especially hyoscine. Holling, McArdle and Trotter (105) found that L-hyoscyamine, in a dose of 0.96 mg., protected 39 per cent of susceptibles against seasickness during trials in which the protection afforded by hyoscine (0.6 mg.) was 53 per cent. It will be recalled that they obtained essentially the same degree of protection by giving 1.0 mg. of atropine (containing 0.5 mg. of L-hyoscyamine). But L-hyoscyamine and atropine were not used on the same trips; each was tested separately against a placebo and hyoscine (0.6 mg.). The results suggested that they are about equally effective and that neither is as effective as hyoscine. Similarly Canadian studies (173, 175) have indicated that L-hyoscyamine is somewhat less potent than hyoscine in preventing swing sickness. There is general agreement, however, that this isomer is fairly effective (67, 105, 175, 182, 210). As regards D-hyoscyamine the only evidence available is that obtained in a single sea trial involving 34 men each of whom was given 2.0 mg. (105). Only 25 per cent of the estimated susceptibles were protected, whereas on the same trip 0.6 mg. of hyoscine protected 75 per cent of the susceptibles estimated to be present in a group of 35. This result suggests that the effectiveness of atropine is chiefly due to its content of L-hyoscyamine, but obviously the potency of D-hyoscyamine as a preventive has not been sufficiently explored. Smith (206) has remarked that the effect of D-hyoscyamine "may not be negligible since there is insufficient evidence that L-hyoscyamine is twice as effective as atropine, as it should be if the effect of D-hyoscyamine is negligible."

d) *Other atropine-like drugs.* Several of the synthetic substances allied to the belladonna alkaloids have been tested (105, 210). They have given little promise of being useful. Employing small groups of individuals susceptible to swing sickness Smith and Hemingway (210) tested homatropine (12 mg.), benzoyltropine (50 mg.) and benzoyloscine (50 mg.) and found these drugs to be at the most only moderately effective. Their usefulness appeared to be definitely less than that of the naturally occurring alkaloids (hyoscine, atropine and L-hyoscyamine) which were also tested in the same group of experiments. Demerol (100 mg.) was found to be devoid of preventive action and in some subjects it caused nausea before they were swung. Pavatrine (250 mg.) when tested on 20 susceptible individuals gave an estimated protection of 42 per cent, but in a later trial involving a larger number of unselected subjects it failed to have any apparent beneficial action. Smith and Hemingway suggest that the substitution of other groups for the tropic acid of hyoscine, atropine and hyoscyamine greatly reduces the effectiveness of these substances as preventives.

e) *Combinations of belladonna alkaloids.* Although hyoscine (scopolamine) seems to be the most effective of the atropine-like drugs, there is some indication that in doses of from 0.8 to 1.2 mg. this member of the series is more apt to produce certain undesirable side effects, particularly those due to action on the 'higher centers,' than L-hyoscyamine (175). Since it is true that the protective effect of these substances is, within certain limits, a function of dosage, attempts have been made to secure optimal action by mixing relatively small quantities of hyoscine with larger amounts of L-hyoscyamine. A commercial proprietary remedy ('Vasano') consisting of the camphorates of L-hyoscyamine and hyoscine was tested during the War on men sub-

jected to the motions of swings (66, 70, 184), boats (28, 103, 105–107, 133) and gliders (180). The estimated protection of susceptibles varied from practically zero to 72 per cent. It has been indicated that this variation was probably due to considerable differences in the alkaloid content of different batches of the preparation (105, 133). When a definite combination of L-hyoscyamine hydrobromide (0.8 mg.) and hyoscine hydrobromide (0.3 mg.) was used, more consistent results were obtained; the percentage of susceptibles protected against swing sickness varied from 35 to 64 (70, 173, 175, 182, 184, 206). To this evidence one may add that secured when this same mixture was given, together with or shortly before 100 to 200 mg. of niacin. This combination was developed in 1943 at Toronto by the Royal Canadian Navy Research Unit and has generally been referred to as 'the R.C.N. remedy.' It was at first thought (184, 203) that niacin augments the beneficial effects of the alkaloids (presumably by increasing the cerebral blood flow), but later work (173, 182) showed that this substance does not add to the effectiveness of other substances and does not alone have any preventive action. The R.C.N. remedy has consistently given good results in tests on swings (173, 182), at sea (132, 185, 220, 221) and in the air (207). From 47 to 73 per cent of the estimated susceptibles were protected in these various trials.

2. BARBITURATES. Certain members of this series of compounds have been incorporated in proprietary remedies. There are several accounts by ship's doctors (87, 99, 158) indicating that one or another of the commonly used barbiturates is effective in seasickness (153), but these observations were not controlled and in some the doses were great enough to produce a degree of hypnosis that is undesirable, especially from a military point of view. In carefully controlled swing tests little or no protective effect was obtained with barbital (325 mg.) (209), amytal (65 mg.) (173), pentobarbital (100 mg.) (203) or seconal (50 and 100 mg.) (203). In one sea trial phenobarbital (65 mg.) had perhaps a slight effect, but another long-acting barbiturate, 'hexobarbitone,' failed to give the slightest evidence of protective action (105). In all these experiments hyoscine gave definite protection. Nevertheless, it should be recorded that amytal in an amount (130 mg.) that produces scarcely any central depression protected 39 per cent of the estimated susceptibles in a group of 76 (28% sick) when the incidence of seasickness was 46 per cent in a control (placebo) group of 70 (220).

The most extensive investigation of barbiturates as possible preventives is one that led to the emergence of a *thiobarbiturate*, ethyl-β-methyl-allyl thiobarbituric acid (V-12), as a moderately effective remedy. This work began in Canada in 1942 and was initiated and chiefly carried on by R. L. Noble (170, 172–174). The basic thought underlying these studies was that it might be possible to find a barbiturate or related compound that would depress the central nervous mechanisms involved in motion sickness and yet would not exert any marked hypnotic or anesthetic effects. Many thiobarbiturates were examined. Toxicity studies on rats, mice and dogs were followed by determinations of the potencies of these compounds as preventives in dogs and human beings (170, 172, 173). In susceptible dogs a large number of barbiturates proved to be very effective (172). Human trials of many of these, however, were precluded by the results of toxicity studies. Others, when tested on human beings, were found either to be ineffective or to exert undesirable side effects (66, 170, 173, 184, 186). The substance designated as V-12 completely protected all susceptible dogs

from swing sickness and did not produce any apparent untoward symptoms (172). The dose required (1.25 to 30 mg/kg.) was directly related to the degree of susceptibility of the animal as previously determined. In the case of human beings V-12 is apparently much less effective, at least when it is given in amounts that produce no undesirable side effects. Noble (173) found in two groups of swing trials that 195 mg. protected 23 and 40 per cent of the susceptibles and in another experiment that 65 per cent were protected by 323 mg. given in a divided dose. The larger amount was slightly more effective than hyoscine (0.65 mg.) or the R.C.N. remedy. Others have not obtained quite such good results with V-12. When Parker (182) gave single doses of 390 mg., only 22 per cent were protected in swing tests. In these same experiments 0.8 mg. of hyoscine protected 73 per cent of the susceptibles. In the sea trials carried out by Tyler (220, 221) 56 per cent of the susceptibles estimated to be present in 1346 subjects were protected by V-12 when the sickness rates in the placebo groups did not exceed 27 per cent and averaged 22 per cent. Three different amounts of the substance were given (260, 325 and 390 mg.), but there was no indication that, within these limits, increasing the dose augmented the degree of protection. In other trials, carried out under various sea conditions, Tyler found that a single dose of 325 mg. gave protection rates of 42, 36 and 30 per cent when the control sickness rates were 19, 28 and 46 per cent, respectively. With the highest rate of sickness among the controls, amytal (130 mg.) was certainly just as useful. These results indicate that V-12 is as effective as hyoscine when the sickness rates in the control groups are below 30 per cent, but that with higher rates hyoscine (alone or in certain combinations) is a better preventive. Noble, however, has obtained evidence that when the daily dose of V-12 (325 mg.) is divided and taken with the morning and evening meals better results may be expected. He states, further, that "continuous treatment starting at least a day before the exposure to motion seems to be advantageous."

3. COMBINATIONS OF BELLADONNA ALKALOIDS AND BARBITURATES. Mixtures of this type were doubtless first introduced with the hope that the two kinds of drugs might act synergistically or supplement each other in their effects on the bodily reactions to motion.

The most extensive tests of a combination of this kind were carried out on a preparation developed before the outbreak of World War II by a medical officer of the United States Army, Lt. Colonel L. L. Barrow. This remedy, known as Motion Sickness Preventive, Army Development Type (MSP) was dispensed in capsules each containing amytal (65 mg.), hyoscine (0.2 mg.) and atropine (0.16 mg.). The usual dose was two capsules. In the case of swing sickness Smith (209), using a small group of 19 men who acted as their own controls, found that only 31 per cent were protected. On the other hand, in air trials he obtained a protection of 52 per cent when he gave this preparation to 176 navigation students (207). The R.C.N. remedy yielded essentially the same protection while hyoscine alone (0.75 mg.) appeared to be a little more effective (67% protected). There is ample evidence that MSP is an effective preventive of seasickness. In the first report concerning it, Barrow, Conn and Williams (28) stated that not one of the 85 men riding in small landing craft who were given MSP became sick. The sea conditions were such, however, that only 10.7 per cent of the controls were sick. A later group of observations, analyzed and reported by

Luykx (132), indicated that 76 per cent of 949 subjects failed to become sick in LST's as a result of taking this mixture. Although the sickness rate in the treated groups averaged 0.8 per cent and that in the control groups (placebo) only 3.3 per cent, the difference appeared to be significant ($P = 0.01$). The much more favorable sea trials which Tyler (220, 221), still later, was able to run off the California coast showed clearly that MSP is quite effective. In this series of studies 2056 men took the mixture 1 to 3 hours before undergoing training operations in small landing craft. Approximately the same number received a placebo and were subjected to the same conditions. In the total of 22 trials the sickness rate in the controls ranged from 5.1 to 53 per cent. At the lowest control rate 92 per cent of the estimated susceptibles were protected, at the highest, 49 per cent. When the sickness rates in the control groups were moderate (e.g., 21 and 28%) the degree of protection was intermediate (67 and 78%, respectively). The average protection was 62 per cent. While Tyler's tests certainly demonstrated that MSP is a good preventive they also showed that hyoscine (0.6 mg.) and the R.C.N. remedy are equally good. They provided no evidence that the addition of amytal enhances the efficacy of belladonna alkaloids. Smith (209) has concluded that the effectiveness of MSP in swing sickness is probably wholly due to its content of hyoscine and atropine. It should be recalled, however, that when the amount of amytal present in the mixture (130 mg.) was given alone a moderate degree of protection resulted (220).

The effectiveness of mixtures of V-12 and one or more belladonna alkaloids has been tested by several workers. In the case of swing sickness Smith (209) found that a combination of V-12 (250 mg.) and hyoscine (0.75 mg.) protected 50 per cent of the susceptibles in a group of 104 subjects, but this degree of protection was no greater than that obtained when 0.75 mg. of the alkaloid was given alone. Parker (182) also concluded that the effectiveness of mixtures of V-12 (290 or 390 mg.), hyoscyamine (0.8 mg.) and hyoscine (0.3 mg.) was no greater than might be expected from their content of belladonna alkaloids. In Tyler's sea trials (220, 221) combinations of V-12 (260 or 325 mg.) with hyoscine (0.6 mg.) or with hyoscyamine (0.8 mg.) and hyoscine (0.3 mg.) were tested on large groups of subjects. The results did not indicate that the addition of V-12 significantly enhanced the protective action of the alkaloids. On the other hand, Noble (173) found that the addition of a relatively small amount of hyoscine (0.4 mg.) to a large dose of V-12 (325 mg.) did not significantly augment the usefulness of the latter as a preventive of swing sickness. When he compared the effects of *a*) 195 mg. of V-12, *b*) 0.4 mg. of hyoscine and *c*) a mixture of the two, he obtained evidence that the combination was more effective than either drug alone. Further, Noble presents data suggesting that when these small doses are used certain individuals are protected by V-12, but not by the alkaloid, and vice versa. This may be an indication that the two kinds of drugs act in different ways, a possibility that is perhaps supported by the fact that hyoscine fails to protect dogs against motion sickness whereas in these animals certain barbiturates are very effective (172).

Although conclusive evidence is lacking that the addition of a barbiturate to an optimal dose of one or more belladonna alkaloids increases the specific action of the latter, there are some reasons for supposing that the use of such mixtures may be desirable (175). The National Research Council of Canada has recommended for use

in the Canadian Armed Services a capsule containing hyoscine (0.1 mg.), hyoscyamine (0.3 mg.) and V-12 (130 gm.). This preparation has been termed the 'Canadian Motion Sickness Remedy—National Research Council Formula' (175). The dose is two capsules taken 2 to 4 hours before exposure to motion and, for continued treatment, an additional capsule every 8 or 12 hours. Certainly this mixture should be as effective as any other that has been tested, and there is evidence that it does not produce any toxic effects when given over a considerable period of time (175, 182).

4. OTHER SUBSTANCES. There have been suggestions that the administration of one or another of the vitamins might decrease susceptibility to motion sickness. Reference has already been made to evidence that niacin has no beneficial action. Noble's results actually suggest that it may have an adverse effect (173). Smith (209) and Noble (173) are in agreement that thiamine is quite ineffective. The use of pyridoxine in the treatment of the nausea and vomiting of irradiation sickness and of pregnancy led to trials of the effect of this member of the vitamin B complex in swing sickness. Given in doses of 100 or 200 mg. it proved to be without benefit (209).

In air or swing trials benzedrine (47, 108, 203, 209), ephedrine (180) and methedrine (105) failed to give any significant degree of protection. When benzedrine (10 mg.) was combined with hyoscine, atropine or hyoscyamine the protection rates were definitely less than those obtained when the same amounts of the belladonna alkaloids were given alone (203). There are two reports, however, of estimated protection rates of 62 and 65 per cent when a mixture of 0.4 mg. of hyoscine, 10 mg. of benzedrine and 100 mg. of niacin was given to men subjected to the motion of swings (184, 203).

The effect of adding prostigmine to one or another belladonna alkaloid has been tested in trials on swings (209) and at sea (220). It was hoped that prostigmine might counteract the undesirable parasympatholytic actions of the atropine-like drugs. Also, there was the claim of S. Wolf (230) that nausea depends on gastric relaxation and that prostigmine may be beneficial because it prevents this visceral change. The experiments of Smith (209) and of Tyler (220) have demonstrated that the protection obtained by these combinations, if any, is small. Further, it is evident that prostigmine decreases the effectiveness of hyoscine (209) and atropine (220) and that a combination of syntropan and prostigmine is quite ineffective (220).

5. PROBLEM OF SIDE EFFECTS. Ideally any drug or combination of drugs employed for the relief of motion sickness should exert its action without producing undesirable side effects. From the point of view of the Armed Services it is most important that the administration of a remedy does not interfere with the ability of the men to carry out their duties.

As regards the belladonna alkaloids it is obvious that in large doses these substances will provoke markedly undesirable effects. These are of two kinds: *a*) a central action, which may lead to mental 'cloudiness,' an excited state or even hallucinations; and *b*) various peripheral effects among which are dryness of mouth and throat, cycloplegia, cardiac acceleration and lessened sweating during exercise or exposure to heat. The question, however, is whether any debilitating side effects develop with appreciable frequency when the atropine-like drugs are given in the small amounts that suffice to prevent motion sickness.

There is abundant evidence that single doses of hyoscine hydrobromide ranging

from 0.5 to 0.75 mg. rarely produce any untoward effect except dry mouth (in about one-third of the cases). Smith and Hemingway (210) found that hyoscine (0.75 mg.), atropine (1.0 mg.) and L-hyoscyamine (1.0 mg.) did not influence pulse rate, blood pressure or the near point of vision, but significantly reduced salivary flow. No significant effects on visual performance were noted in navigation students on long flights after they had received 0.6 to 0.75 mg. of hyoscine (128, 207). Keil (122) showed that oral doses of hyoscine greater than 1.5 mg. produce effects on accommodation which significantly lower visual efficiency in 50 per cent of the individuals tested. This amount of the drug, however, is more than twice the dose effective in preventing motion sickness. More pertinent are the results obtained by Keil after 1.0 mg. had been given: of 15 subjects 3 showed slightly lessened acuity, 3 greater acuity and 9 no change. As regards the effect of hyoscine on marksmanship, the most thorough study is that of Corey and Webster (59). The subjects were 60 U.S. Marines who had been firing for 3 days prior to the beginning of the experiment. They continued to fire during the next 2 (experimental) days, after being divided into 2 groups equal in numbers and in demonstrated ability with the rifle. The members of one group received an initial dose of 0.65 mg. followed by 0.32 mg. every 6 hours thereafter during a period of from 24 to 48 hours; the members of the other group received placebos. The placebo group showed a superiority over the hyoscine group (of about 8% hits) that is statistically significant, but this superiority "was expressed as an increased fire power above the pre-experimental norm, while men receiving scopolamine performed with equal efficiency before and after the administration of the drug." The amounts of hyoscine given in these tests produced no untoward symptoms aside from a feeling of sleepiness, some dryness of the mouth and suppression of lacrymation. In a study involving larger groups Tyler (221, 222a) found that the R.C.N. remedy (0.8 mg. hyoscyamine, 0.3 mg. hyoscine, and 200 mg. niacin) had no effect on the marksmanship of either unselected or highly skilled military personnel. The difference between the results obtained by Tyler and by Corey and Webster may have been due to the fact that the former gave a single dose 2 hours before firing began while the latter investigators administered the alkaloid every 6 hours during the 2 experimental days. It is also possible that the predominance of hyoscyamine over hyoscine in the R.C.N. remedy contributed to the difference in results.

When Holling, McArdle and Trotter (105) raised the dose of hyoscine from 0.6 to 1.2 mg. the only side effect noted was dryness of the mouth; drowsiness and eye symptoms were not encountered. Further, they reported that neither this amount of hyoscine nor 0.96 mg. of L-hyoscyamine had any obvious effect on physical performance, ability to shoot or near vision. On the other hand, Canadian investigators (175) have reported that doses of from 0.8 to 1.2 mg. of hyoscine may produce a change in heart rate, dry mouth, cessation of sweating and, quite commonly, a sensation of 'cloudiness.' They found that with similar doses of L-hyoscycamine the unpleasant mental effects were absent, although some dryness of the mouth and occasional visual disturbances might occur. Accordingly the R.C.N. remedy (a mixture of 0.8 mg. hyoscyamine and 0.3 mg. hyoscine) represented an attempt to keep the amount of hysocine as low as possible and to augment the therapeutic effect by adding hyoscyamine. When this mixture was administered as an initial dose and half-

quantities given thereafter at 10-hour intervals for 40 hours the only adverse symptom obtained was dryness of the mouth (in 40 to 50 % of the subjects); there were no effects on physical or mental performance. A careful study by Birren, Stormont and Fisher (36), in which a battery of tests covering a variety of sensory and motor tasks were employed, revealed that neither the R.C.N. remedy nor two other effective mixtures containing belladonna alkaloids exerted any significant untoward actions; again, about half experienced dryness of the throat. There is agreement that the diminution in sweating produced by even the larger doses of atropine-like drugs is not sufficient to contraindicate their use in hot climates (105, 175).

It has been pointed out above that in general the barbiturates commonly used in clinical medicine are not effective preventives. Further, it is obvious that even in moderate doses they are apt to produce a degree of hypnosis that is undesirable, especially in the case of military personnel. There is, however, some evidence (220) that amytal, given alone in an amount (130 mg.) that evokes little obvious central depression, is a mildly effective preventive (220). Yet it appears that the addition of this same amount to small effective doses of belladonna alkaloids, as in MSP, does not enhance the prophylactic efficacy of the preparation (see above). The 2 grains of amytal doubtless adds to the tendency of the alkaloids to produce drowsiness. This action is certainly one to be avoided when individuals are required to remain alert for long periods of time, as on long flying missions. On the other hand, under conditions of tension and excitement, e.g., during amphibious or airborne landing operations, the addition of a sedative to a motion sickness preventive may be highly desirable. Indeed it has been demonstrated (222) that barbiturates (amytal or V-12) can be given to men who stay awake for over a hundred hours without significantly affecting their performance or their ability to stay awake. In this connection attention should be directed to a study (221, 222a) which indicates that the amytal content of MSP is probably responsible for the fact that marines who received this medication achieved better scores than their fellows who fired after receiving either the R.C.N. remedy or a placebo.

In his extensive study of the thiobarbiturates Noble paid much attention to the side effects of these substances. He and others have carried out toxicological experiments on animals and have tested on human beings those members of the group which in dogs proved to be at once relatively effective and of low toxicity (170, 175). Here it will suffice to confine attention to V-12 (ethyl-β-methyl-allyl thiobarbituric acid) which, as indicated earlier, has proved to be the most useful of these compounds. Noble (175) points out that thiobarbiturates in large doses may cause liver damage and that administration of compounds closely related to V-12 has been followed by the sudden onset of fever and agranulocytosis. Yet human subjects who received 315 mg. of V-12 daily for 30 days showed no symptoms and liver function tests remained normal throughout. The immediate symptom of overdosage is sleepiness. On the other hand, when 315 mg. was given as a single or divided daily dose only a small number of subjects became drowsy; this amount appears to be well tolerated by the majority of individuals. Tyler (221, 222a) has shown that a single dose of 260 mg. has no effect on the rapid fire marksmanship of 'expert' riflemen.

APPENDIX

'Dramamine' as a Preventive and Curative Agent. Just after this review had been completed there appeared three reports that β-dimethylaminoethyl benzohydryl ether 8-chlorotheophyllinate is effective in both the prevention and cure of motion sickness. This compound, supplied under the trade-name 'Dramamine,' appears to have value in the control of certain allergic manifestations; it possesses antihistaminic properties and has some antispasmodic action.

Gay and Carliner (*Science* 109: 359, 1949; *Bull. Johns Hopkins Hosp.* 84: 470, 1949) were the first to test this substance as a therapeutic agent in motion sickness. Their reason for doing so is interesting because it shows how a purely incidental observation may serve as the basis for provocative studies in an entirely new direction. They write: "Among other patients, the drug was administered to a pregnant woman with urticaria who incidentally had suffered all her life from carsickness. It was possible to control the car sickness of this patient at will. A placebo failed repeatedly, but Dramamine gave complete relief if she took 50 mg. a few minutes before she boarded a streetcar." After some preliminary observations on susceptible individuals they carried out a study on troops during the transatlantic passage of an Army Transport. The results obtained led these observers to believe that this substance is "more uniformly effective in the prophylaxis and treatment of motion sickness than any drug so far employed." Since none of the drugs previously found to be of use in the control of motion sickness appears to be universally effective, one welcomes the introduction of a new and promising agent. But a critical examination of the report by Gay and Carliner does not permit the conclusion that Dramamine is more effective than other preparations in the *prevention* of motion sickness. Even their testimony that it almost always effects a dramatically rapid cure in the violently sick may be subject to some questioning on methodological grounds. We refer below to three aspects of the study which seem pertinent in the determination of its reliability and which bear on the question whether there is at hand any evidence to show that Dramamine is actually superior to other remedies.

1. The subjects were divided into experimental groups on the basis of compartments occupied. This is hardly a sound procedure, for it is clear that even in adjacent parts of a ship men may not be subjected to conditions that are comparable. It is significant that during the first 12 hours the incidence of sickness in subjects, who received neither a placebo nor the drug, was 11.6 per cent in compartment 4-E and 33.3 per cent in compartment 4-F. In the only prophylactic test carried out on the entire trip—it ran during the first 48 hours—123 men in compartment 3-F served as the placebo controls for the 134 in 3-E who received Dramamine. Since the spatial relationship of 3-E to 3-F was the same as that of 4-E to 4-F, it is quite possible that a similar difference, one wholly referable to space, was a significant factor in producing the difference in incidence between the occupants of compartment 3-E (who received the drug) and the men in 3-F (who were given a placebo).

2. It has been demonstrated repeatedly that a rigorous evaluation of a preventive requires that it be tested under conditions in which the sickness rates in the control groups range from moderate to severe. Many preparations give excellent protection as long as the incidence in the controls remains low or moderate (10 to 30%), but with high sickness rates (50% or more) their effectiveness falls off and, in the case of certain barbiturates, may be almost completely lost. In this case the prophylactic trial occurred during the first 48 hours and involved a treated group of 134 men in compartment 3-E and a placebo group of 123 in compartment 3-F. In the former no individual developed nausea or vomiting and 2 complained only of 'dizziness.' In the control group 35 men (28.4%) experienced nausea or vomiting within the first 12 hours and apparently no others developed symptoms during the 2-day period of placebo treatment. This is not a high incidence. If we consider also the sickness rates in the untreated groups of compartments 4-E (11.6% of 129 men) and 4-F (33.3% of 99 men) during the first 12 hours, the overall average is 23.6 per cent. Assuming that all these figures are statistically reliable and counting the 2 cases of dizziness in the treated group as sick the percentage of susceptibles protected by Dramamine was 94 per cent. With such moderate sickness rates in the controls both hyoscine and MSP will on occasion give protection rates as high as 80 per cent. However, as the sickness rates in the controls increase the effectiveness of these remedies falls off. That this is also the case with Dramamine is indicated by the report of Strickland and Hahn (*Science* 109: 359, 1949) on the prevention of airsickness by this drug. Under well controlled conditions in

which 55.6 per cent of those receiving placebos became sick 28.7 per cent of the treated individuals were also sick. Here only 48.4 per cent of the susceptibles were protected. Hyoscine has been shown to give this degree of protection under similar conditions. In view of these facts, and in view of the fact that Dramamine has not yet been tested simultaneously either with hyoscine or any other established preventive, it is premature to claim that this drug is more effective than any other substance in the prophylactic control of motion sickness.

3. The reports of Gay and Carliner concerning the alleviation or total cure of severe cases of seasickness by administration of Dramamine are impressive. In these therapeutic trials, however, two uncontrolled factors may have influenced the results to some extent. Control groups did not continue to receive placebos or remain untreated during the periods of observation of those who received the drug. Many subjects who became sick were alternately placed on Dramamine and a placebo. Therefore it is quite impossible to determine to what extent changes in the effectiveness of the ship's movements contributed to the remission of symptoms. For the same reasons it is impossible to ascertain the influence of adaptation which may be great in any continued exposure to motion. That adaptation occurred in many of the men is indicated by the fact that of the 33 in compartment 4-F who became sick within the first 12 hours and then received a placebo 19 (58%) recovered within 2 days and remained well during the rest of the voyage although the lactose treatment had been discontinued. One naturally wonders how many of those who became sick in compartment 4-E and then received Dramamine throughout the voyage owed their lasting improvement to the process of adaptation. Strong evidence that many subjects experienced adaptation is afforded by the fact that of the 187 men in another experimental group who were quickly relieved of severe symptoms by Dramamine, 143 (75%) failed to become sick again when the drug was discontinued. In this connection it is significant that the weather was roughest during the second half of the voyage. Although it seems likely that the influence of adaptation and changes in motion contributed to the overall apparent success of the treatment, Dramamine certainly effected many rapid and complete cures of severe cases of sickness. In 300 instances of sickness in which the drug was given complete relief was obtained in 288 (96%). Whether this substance is superior in this respect to other substances which have been shown to be effective preventives is a question that remains undetermined, for no comparative results are available.

No studies of side-effects have been reported. It seems likely that the substance would induce a certain degree of drowsiness.

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ZINC IN THE MAMMALIAN ORGANISM, WITH PARTICULAR REFERENCE TO CARBONIC ANHYDRASE

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ZINC IS AMONG THE ELEMENTS grouped together as 'trace metals', a misleading designation which implies that these substances can be identified qualitatively but cannot be measured quantitatively. It has always been difficult to determine whether the occurrence of these elements in biological material is accidental or is indicative of physiological function. If the latter was established or suspected, it still remained to be determined what the physiological role of the element was.

Lack of progress in this field was due primarily to the slowness of development of chemical and physical techniques accurate for estimation of the minute quantities of the elements concerned. Some methods suggested were inaccurate or invalid, and so gave rise to divergence in the results of investigators working at different times. The serious limitations of earlier methods must be borne clearly in mind in the evaluation of the significance of the findings of their users. Similarly, biological experiments based on deprivation in animals have been most difficult to carry out because of the very minute quantities in which some elements may be active in living organisms. Since occurrence of such elements is wide-spread, the preparation of synthetic diets free of them is a most difficult problem, and almost insoluble in animals of the higher species.

It has become evident in recent years that zinc is important in human physiology, and accordingly findings of observations in animals having a direct bearing on this problem will be reviewed here. Previous studies of the biological activity of zinc have emphasized its toxicological aspects, e.g., metal fume fever. This subject, which has been adequately treated elsewhere (60), will not be discussed.

ZINC CONTENT OF TISSUES AND BODY FLUIDS

Tissues. The zinc content of various tissues has been studied by many workers during the past forty years. Various investigators have attempted to determine the absolute quantities of zinc in plants, invertebrates, and the organs of vertebrates including man as a possible clue to its function (10, 23, 36, 42, 45, 62, 83, 98, 126, 137,

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161, 173, 176). The diversity of methods used by these various investigators attests to their inadequacy. On the basis of recent polarographic work and the use of the sensitive dye diphenylthiocarbazone and related compounds, it has become apparent that the findings of investigators working before their development must be discounted largely as inaccurate from a quantitative viewpoint. Furthermore, comparison between these earlier results is complicated by the fact that units chosen to express concentrations vary markedly. Data are reported per gram or kilogram of wet and dry tissue and since these are not absolute standards of reference comparison is difficult with data expressed in terms of grams of ash. In no instance were the organs perfused to free them of blood present, or corrections made for this source of error. It would be desirable that future work would refer the tissue content of zinc to relatively stable biological constituents, as nitrogen, which could serve as standards of comparison. Arbitrarily choosing those data referring to wet weight, it is obvious that zinc is present in all human and other vertebrate organs in quantities varying from 10 to 200 μg/gm. wet weight (83, 98, 166). Most organs including the pancreas contain between 20 to 30 μg/gm. Liver, voluntary muscle and bone, contain about double this amount while the endocrine organs and lymph nodes contain from 60 to 180 μg. Large quantities of zinc have been found in the corneal epithelium, the iris, retina and lens (98, 161). These data deserve critical re-examination on a larger scale than has heretofore been attempted.

The distribution of $_{30}Zn^{65}$, a radioactive isotope of zinc, in the mouse and dog, has been studied (118, 144, 145). Animals were serially killed after injecting the isotope, and following sample preparation, the investigators counted the gamma-rays. The amounts of Zn introduced were negligible as compared with the total amount present in the body. Tissue content of $_{30}Zn^{65}$ was expressed in terms of percentage of the injected $_{30}Zn^{65}$ found in each organ. No effort to perfuse the organs was made, and the data are not corrected for blood content.

In both mice and dogs the liver contained the largest fraction of the injected dose early in the experiment. There was a gradual decrease of the activity in both species which was more rapid in the mouse than in the dog. The most rapid uptake and loss during the period of study of 160 hours was observed in the liver, pancreas, kidney and pituitary. Least activity was found in the erythrocytes, brain, skeletal muscle and skin. The activities of spleen, gastro-intestinal tract, adrenals, lungs, lymphnodes, bone, heart and thymus were intermediate. It should be noted that red blood cells and bone were the only tissue components which *accumulated* zinc. Unfortunately, the authors did not measure the total zinc content of organs along with their radioactivity content. Therefore, these data are not significant in regard to over-all zinc metabolism. The ratio Zn^{65}/Zn stable would be a far more meaningful parameter than either measurement alone. While the observations of these authors only extended over 160 hours, it has been shown (165, 166) that the isotope could be detected in dogs, normal humans and one patient with myelogenous leukemia (58) for as long as 8 months, and probably a year. In these studies there was no evidence of selective uptake of Zn^{65} in any organs anywhere.

Injection into a pregnant dog demonstrated the passage of isotope across the placenta into all of 7 puppies (166). The total stable zinc content of the tissues of

these animals was similar to that of adults, though the ratio of radioactive to total zinc in 3 of these animals killed at intervals during the first postnatal week showed a re-distribution of the isotope in all organs: there was a decrease in concentration in the extremities, and a rise in concentration in the viscera (166).

Blood. The literature contains few references to the quantitative determination of zinc in the blood of normal humans and higher vertebrates (21, 23, 36, 81, 85, 101, 146, 168). A comparison of these results is complicated again because of the different methods used and the different units chosen. Using polarography with an accuracy of 2 to 3 per cent (146) the following mean species differences per gram of erythrocytes have been found:

Man 13 μg/gm.	Rabbit 9 μg/gm.
Rat 10 μg/gm.	Goose 6.5 μg/gm.
Dog 9 μg/gm.	

Recent work utilizing a diphenylthiocarbazone technique (167,170) accurate for 1 μg. with a standard deviation ±0.1 μg. established human statistical norms in terms of commonly used clinical units as follows:

WHOLE BLOOD 1 cc.	ERYTHROCYTES 1 cc. packed	PLASMA 1 cc.	*WBC*
8.8 ± 2.0 μg.	14.4 ± 2.7 μg.	3.0 ± 1.6 μg.	
	MILLION CELLS		MILLION CELLS
	$1.3 \pm 0.20 \times 10^{-3}$ μg.		$3.2 \pm 1.3 \times 10^{-2}$ μg.

The zinc content of the cytoplasm and nuclei of the goose, turtle, frog, and fish—all animals having nucleated erythrocytes—has been examined (81, 117). The investigators found about 3 times as much zinc in the separated nuclei as in the cytoplasm. The findings for plasma and erythrocytes are as follows:

	TOTAL BLOOD	PLASMA	RBC	ERYTHROCYTES / PLASMA
	Zn	*mg/kg.*	*wet*	*wt.*
Goose	9.8	2.3	7.5	3.2
Turtle	14.2	4.4	9.8	2.3
Frog	22.2	8.5	13.7	1.6
Fish	7.3	4.6	2.7	0.6

This showed an increase in the erythrocyte/plasma ratio with phylogenetic evolution, probably a consequence of the accumulation of carbonic anhydrase in erythrocytes (81).

There is no variation in zinc levels with age or sex. Three-quarters of the blood zinc was found in erythrocytes, 22 per cent in plasma and 3 per cent in leukocytes (168). Though only 3 per cent of the total blood zinc is contained in leukocytes, cell for cell they contain twenty-five times as much zinc as do erythrocytes. The data available do not disclose the possible differences in the zinc content of the various classes of leukocytes. The absence of uniform distribution in the various components of blood suggests that zinc is a physiological constituent of blood; its variations in concentration follow the mathematical pattern of commonly observed biological distribution phenomena.

The nature of the bond between plasma proteins and zinc is not understood at

present (155). Fraction IV-17 has been shown to bind zinc in proportions of 7.4 μg/gm. of protein at *p*H 7.8; and 3.8 mg. zinc/μg. at *p*H 7.0 (166, 171). However, it is highly doubtful that this is a physiological transport mechanism.

The discovery of zinc in relatively large amounts in leukocytes is surprising, but the role of zinc in leukocytes is completely unknown and there are no clues as to its possible function. Attempts at radioactive tagging in order to study the leukocytic life span have at best been inconclusive (164). The possible occurrence of exchange of zinc across the white cell membrane contributes to the difficulties of interpretation, and, most important of all, the nature of the compound with which zinc is associated in the leukocyte is unknown at present.

Sperm. Mann (105) has investigated the iron, zinc and copper content of ram semen and found the following comparative distribution:

	SPERM	PLASMA
	mg/100 ml. semen	
Fe	.68	.16
Zn	.70	.28
Cu	.12	.05

Excretion and Absorption. In 1927, Drinker, Fehmel and Marsh (39) reported on the zinc content of normal human urine and feces. The data were obtained with a method of questionable validity, but they seemed to indicate that 1 mg. was excreted in 24 hours in the urine and about 10 mg. in the feces over a similar period. There was a rise in fecal excretion when zinc-rich foods, such as oysters, were consumed. No concomitant changes in urinary zinc excretion occurred. Apparently, the excess zinc was passing through the gastro-intestinal tract without being absorbed.

McCance and Widdowson (108) state that the normal human intake is 10 to 15 mg/day, most of which is excreted in the feces; the urinary output appears to be independent of intake. No actual figures are available which define the minimum daily requirement in man with precision. McCance and Widdowson found it striking that iron and zinc vary together in almost all foodstuffs, i.e., substances having large quantities of one have a similarly high content of the other, and conversely.

By means of intravenous injections they found that most subjects react to an overdose by gastro-intestinal excretion, though individually different physiological reactions occur which may be related to the state of tissue zinc saturation. They found the normal urinary zinc per day to be 300 to 400 μg. while in albuminuria it rose to 2.1 mg/day, a seven-fold increase. This loss was not compensated for by diminished fecal excretion. Similar increases in zinc in nephritic urines had previously been found (48), though there was no correlation with the degree of albuminuria. McCance and Widdowson (108) found 5.1 to 10.3 mg/day in feces, depending upon intake.

Milk. The quantities of zinc in human, cow and goat milk were investigated (82). The data were obtained by polarography. It was concluded that colostrum has a high zinc content and that in women the zinc concentration falls to 2 mg/kg. of fresh milk during lactation. Definitive studies on zinc utilization with Zn^{65} are indicated and would help to solve many of the questions posed.

PRODUCTION OF ZINC DEFICIENCY IN ANIMALS

In 1922 an interesting series of experiments was initiated (11) by depriving mice of dietary zinc. The difficulties inherent in designing a diet deficient in such a widely distributed element which would simultaneously supply all other essential nutrients were realized. The authors were aware that their diets probably lacked vitamins, the nature of which was not known at the time. In spite of the obvious experimental defects they observed that the controls receiving zinc lived longer than the zinc-deprived animals. However, both groups died (12). Similar findings were obtained by other authors (70).

The experiments were repeated some ten years later (13, 14) with diets improved by the addition of vitamins. The young were separated from their mothers at 21 days. Their zinc-free diet was admitted to contain more than 50 μg. of zinc/100 gm. of diet, but these data are questionable. The animals on this diet succumbed within 14 to 23 days. They failed to grow, lost weight and died with symptoms of ataxia and posterior column disease. The control animals receiving zinc survived, on the other hand, for 57 to 74 days. The authors concluded that zinc is essential for growth and survival of the normal mouse.

Almost simultaneously, similar results were obtained (162) though these workers concluded that the diets were inadequate in other respects as well. These results were contrary to earlier inconclusive results of other workers, probably as a result of more thorough purification of diet (121).

The Wisconsin School has carried these investigations forward, adopting the rat as an experimental animal. By close attention to preparation of diets these workers were able to obtain normal growth of the control animals. The growth of zinc-deficient rats was only one-third as good as that of normally fed ones. The fur softened and turned from black to gray in 6 to 7 weeks. The zinc-deficient animals required 52 per cent more ration to gain a gram in weight. Two animals which were fed zinc adequately after termination of the experiment returned to normal appearance (154). Further work (66) showed that the zinc-deficient rats received 22 μg/day of zinc. Feeding of 30 μg/day of zinc caused marked improvement and 40 μg/day completely removed zinc deficiency manifestations. The controls received 300 μg/day.

Studies of the carbohydrate metabolism in these zinc-deficient animals showed the glucose tolerance curves to be delayed and irregular; there was no glycosuria, however, and the blood sugar was normal, as was the liver glycogen. These findings suggest the occurrence of delayed absorption of glucose from the gastro-intestinal tract (66). The protein metabolism was disturbed also, as evidenced by a distinct delay in the time required for the blood nitrogen to reach a maximum, and by the lower plasma total protein level in the Zn-deficient rats. The efficiency of food conversion to body tissue was much increased by pituitary transplants (66).

Additional work was concerned with the possible effect of zinc deficiency on enzyme activity as causes of the observed disturbances in carbohydrate and protein metabolism. There was a definite lowering of pancreatic amylase and proteolytic activity in zinc-deficient animals which, however, could not be restored by the addition of zinc *in vitro* or *in vivo*. Similar results were obtained in magnesium deficiency and the change was considered to be the result of general debility (67). Zinc had no effect on crystalline pepsin and trypsin. While no changes in bone phosphatase were

found, intestinal phosphatase activity in zinc-deficient rats was considerably decreased, a finding which was regarded by the authors as a possible explanation for the disturbed amino acid metabolism described. In all the animals zinc content of the soft tissues was normal, but that of the bone was lowered (68). The fact that zinc is part of carbonic anhydrase (see below) led Hove *et al.* (69) to study the state of this enzyme in zinc-deficient animals. Though there was a slight anemia, no striking lowering of the enzyme activity was found.

The uric acid content of zinc-deficient rat's blood was found doubled on all diets tested (182). Carbohydrate, fat, protein or nucleoprotein content of the diet do not influence the course of zinc deficiency but the uric acid blood findings are not reversed until 5 weeks after zinc has been added to the deficient diet. This increased uric acid content seemed to be due to an increased uric acid formation, not lack of destruction since uricase activity was completely normal.

Work by Day and McCollum (33) essentially confirms these findings. These workers found retardation of growth in zinc-deficient rats after 2 to 3 weeks. They noted marked eczema in two cases and alopecia in a few rats. The alkaline phosphatase of the blood was markedly reduced but no changes in the kidneys or bone were found.

Carbonic anhydrase activity per unit of erythrocytes was unchanged. Later work by Day in mice (32, 34) showed that zinc deficiency caused a decrease of liver catalase activity which could not be restored by addition of zinc *in vitro*. Liver esterase and riboflavin in heart, lung, kidney and liver was unchanged. Cadmium did not replace zinc in deficient diets. It was found that zinc was one of the causes of sterility in heifers, which could be remedied by zinc oxide administration (119).

A histological study in zinc-deficient rats (33, 57) revealed hyperkeratinization, thickening of the epidermis, intra- and intercellular edema in the skin and mucous membranes of the esophagus and mouth.

Two balance studies concerning the retention of zinc in pre-school and school children respectively (143, 152) add little to the information on fecal and urinary excretion reported by McCance and Widdowson, and the character of the data presented is such that no general conclusions can be drawn.

The only reports on clinical human zinc deficiency in blood and tissues come from the painstaking work of Eggleton (40–42). In Chinese subjects affected with subacute and chronic beri-beri he found the zinc content of toe- and fingernails and skin reduced to half the normal value. The blood zinc was subnormal compared to the author's normal controls. Similar blood zinc changes occurred in nutritional edema and semi-starvation. On the contrary, the liver and kidney zinc and copper contents were increased in similar cases. No changes were encountered in diabetes mellitus. This work, however, is as yet inconclusive and fragmentary. Zinc is indispensible in mouse and rat physiology, and several human pathological states are accompanied by or may be related in some way to zinc deficiency.

ZINC INTOXICATION

No attempt will be made to cover the pharmacological and chemical changes in man or animals given zinc, except to point out that the intravenous administration of 2 mg/kg. of zinc gluconate is tolerated well by both dog and man (166). Four

mg/kg. in the dog produces lassitude, decreased tendon reflexes, bloody enteritis and diarrhea and paresis of the hind legs. The electrocardiographic changes are similar to those observed in potassium intoxication (166).

Doses of 300 mg. of zinc gluconate daily were tolerated without apparent ill effect by a patient with myelogenous leukemia. Doses of 15 mg. intravenously in a dog weighing 15 kg. depressed the leukocyte count temporarily and one dose of 100 mg. in a patient with myelogenous leukemia was followed by a fall in WBC count from 300,000 to 150,000 over a period of five days, after which it rose to the previous level. Further intensive therapy totaling about one gram, failed, however, to affect the disease process permanently (58).

There is a series of observations on the effect of injected or ingested zinc on metabolic processes which deserve attention (37, 72).

In a series of cats fed large doses of zinc, Drinker, Thompson and Marsh (38) observed three which developed fibrosis of the pancreas. These findings were confirmed (137). Following completely negative results (61) in rats which were fed a diet containing 0.25 per cent of zinc it was reported (120) that doses as small as 0.1 mg/day of zinc, fed together with iron, assisted hemoglobin regeneration in the rat. One-half a milligram of zinc, however, retarded regeneration. Sutton and Nelson essentially confirmed these findings (157, 158, 160) and noted that rats fed 0.5 per cent zinc as the carbonate per day showed impaired production of erythrocytes which could be restored by termination of the diet; anemia and retarded growth resulted. The largest dose tolerated apparently was between .5 and 10 per cent of zinc of the total diet per day. On the latter diet animals died after four weeks. Terminally, the authors detected an excess of leukocytes and immature erythrocytes in every case. It should be stated clearly, however, that these quantities of zinc given daily are far above the likely physiological consumption of the element. Similarly, the production of glycosuria (159) by means of high zinc intake by stomach tube is not likely to occur under normal conditions.

An interesting paper confirmed these findings (148). It was shown that rats fed a diet containing one per cent of zinc developed a severe anemia in 3 to 5 weeks, ceased growing, and many died before the sixth week. On a diet containing 0.7 per cent a severe microcytic, hypochromic 'zinc anemia' was produced in four weeks, but the animals lived longer. The additional feeding of copper maintained the hemoglobin at statistically significantly higher levels, and a mixture of iron, copper and cobalt maintained the hemoglobin at or close to normal levels. These elements had no effect on the subnormal growth of the animals fed zinc, but supplements of a liver extract produced a significant growth response. The feeding of zinc brought about a substantial leukocytosis which was almost double of normal when supplementary liver was fed. These findings were of great interest in view of the recent findings that zinc is an integral part of leukocytes and erythrocytes and possibly is related to the maturation of both these cellular elements (168).

ZINC IN PATHOLOGICAL CONDITIONS

Cancer. Interest in the relation of zinc to cancer developed as a result of the reports (36) concerning the occurrence of zinc in many human and vertebrate organs

and that certain tumors were particularly rich in zinc (28–30, 85). On the basis of all present biochemical and chemical knowledge of the techniques used for preparation and analysis of samples studied, all of this work must be regarded as invalid and of value only insofar as it may have stimulated interest in this field. None of this work will. therefore, be considered further.

Michailowsky reported the experimental production of teratomata in the cock's testis bv injection of zinc chloride (114, 115). He noted that this tumor rarely occurs spontaneously in these animals. Periods of high normal or induced sexual activity seemed necessary for the effectiveness of these injections (6). The results were confirmed (4) by transplantation of these tumors, and by identical results with zinc sulphate and copper salts (6, 49, 50–52). It is highly questionable that these data have any physiological significance.

This work prompted Bischoff (16) to investigate the possible carcinogenic role of zinc, which is so frequently injected in man contained in protamin-zinc insulin. Using the Marsch-Buffalo strain of mice, bearing inbred adenocarcinomata of the breast, he found that zinc injections into the breast locally actually inhibited the development of carcinoma as compared with injections of normal saline into controls. The significance of these unconfirmed results is not clear. In two papers published in 1939 and 1940 it was reported (155, 156) that malignant tissues have a very high zinc and very low copper content as measured by means of a polarographic method. Rabbits inoculated with sarcomata showed an increased zinc concentration in the tumor as well as in all other tissues, serum and urine. Clinically, similar findings were encountered in patients.

Recent work on carcinomata and sarcomata of the stomach, colon, breast and prostate do not confirm these findings. Rather, the values were slightly lower than normal though the number of specimens examined was not large enough to lend itself to statistical evaluation. The zinc content of leukemic leukocytes has been found to be depressed to a tenth of the normal value (58). Under successful therapy with x-ray and urethane the zinc content of leukocytes rose to normal levels as the leukocyte count fell. Attempts at influencing the disease process by injection of stable zinc compounds (see above) failed to influence the disease process or raise the zinc level of the leukemic cells.

The lowered zinc content of leukemic leukocytes is of interest when compared with data (22) on mouse epidermis painted with methyl-cholanthrene to induce hyperplasia and carcinogenesis. These workers report the ratio of the metal content to nucleoprotein-bound phosphorus in normal skin, epidermis, and again following carcinogenesis. The copper nucleoprotein-bound phosphorus ratio was lowered to 17 per cent of normal and the zinc nucleoprotein-bound phosphorus ratio was lowered to 32 per cent of normal. Similarly, there was a marked decrease in iron and calcium. Seemingly, a new chemical equilibrium was established. The studies on leukemia were only concerned with one metal variable while the work of Suntzeff and Corruthers shows that under similar circumstances in carcinogenesis a rearrangement of the pattern of the cellular metals takes place.

It seems apparent from these studies that the neoplastic cell may have a metal distribution quite different from the normal. Which is cause and which is effect is

impossible to conjecture upon at this juncture. Certainly, these considerations deserve attention in view of the changes which take place in distribution of enzymes in neoplastic cells.

Other Pathological Conditions. A report by Scott and Fisher on the zinc and insulin content of normal and diabetic pancreas has evoked much controversy (55, 56, 137, 138). These authors reported that the mean zinc content of normal pancreas from 14 instances of fatal accidents was 0.14 milligrams of zinc per gram of tissue and the mean insulin value 1.7 IU/gm. of tissue. They stated, on the other hand, that the pancreas in 18 diabetic subjects contained 0.07 mg. of zinc per gram of tissue and 0.1 to 0.5 IU of insulin per gram. The authors concluded that the diabetic pancreas contained a quarter the normal amount of insulin and half the normal amount of zinc and a correlation between the two parameters was implied. A spectrographic technique was used, the details of which are not available, but data on zinc contents appear three to six times too high by comparison with results obtained by more recently developed techniques.

These investigations prompted Eisenbrand and Sienz (45) to examine the subject with a dithizone technique (131, 132). Dry and wet weights as well as the fat-free gland were used as internal reference standards. The non-diabetic mean zinc concentration was 30.6 μg/gm. of fat-free gland. The diabetic mean zinc concentration was 25.3 μg/gm. fat-free gland. These values are compatible with the findings of other authors. There was much variability in the data in the so-called normal and diabetic groups. This apparent difference was caused by some sporadically high findings in the control group which raised its mean value. Unfortunately, the control group was heterogeneous and hardly any cases were included which were really normal. On the basis of statistical analysis the authors concluded that the difference between 'normal' and diabetic pancreas could not be considered significant. They decided that there was no discernible physiological relationship between zinc and insulin, and could not confirm the contention that the pancreas zinc concentration was exceptionally high as compared with other organs, or that diabetic pancreas showed zinc concentrations different from normal pancreas (45). An attempt of Horvai to study the influence of hypophysectomy on the zinc concentration of the pancreas permits no conclusions (65).

It has been found recently that the zinc content of erythrocytes of patients affected with pernicious anemia was markedly elevated before therapy with liver was instituted, out of all proportion to the increase in cell size (163, 164, 169, 172). After institution of therapy there was a continuous fall of the zinc content which reached normal levels in 60 to 70 days, a period of time which has been shown to be the mean life span of the red blood cell in pernicious anemia. These data were, therefore, considered to indicate that the erythrocytes in pernicious anemia were gradually replaced by normal cells formed under the influence of liver therapy. It was concluded that iron and zinc deficiencies probably run parallel and probably occur concommitantly.

There is as yet no explanation for the observed elevation of leukocyte zinc in 'refractory anemias' with leukopenia (169).

ZINC COMPLEXES

Hormones. The crystallization of insulin as a zinc salt (133) led to the belief, now widely held, that zinc is an integral part of the insulin molecule. This belief persists in spite of the fact that nickel, cobalt and cadmium can be used instead of zinc in the crystallization (133). Actually Scott and Fisher never stated that zinc was an integral part of the insulin molecule. They merely reported that the metals and insulin combine in a constant ratio. For zinc the percentage in combination was 0.52, for cadmium 0.77, for cobalt 0.44; these percentages were not changed by re-crystallization (134). Moreover, amorphous insulin is as active physiologically as crystallized insulin, and there is no evidence at present that zinc and insulin must combine *in vivo* to form an active compound. There is far more zinc in pancreas than would be necessary for insulin activation (56).

The quantity of zinc in crystalline insulin has been found to be 0.355 per cent (107) while others found it to vary from 0.33 per cent at *p*H 5.06 to 0.65 per cent at *p*H 6.53 when the ionic strength was varied (26, 27). Seemingly, no more than two atoms of zinc have thus far been found to be bound by an insulin molecule. The various mechanisms of the binding of zinc to insulin have been studied, and it was concluded that it may take place either in relation to an NH_2 or to a COOH group (44). If all 29 to 37 COOH groups known to exist in the molecule were to be saturated with zinc the content of the latter would be between 2.7 and 3.5 per cent, a much higher range than that reported by all workers. However, depending on the number of COOH and NH_2 groups participating in chemical zinc binding different results of the percentage of zinc in insulin may be expected.

Understanding of the interrelation between zinc and insulin has been further complicated by the finding that 0.02 per cent zinc added to insulin as the chloride reduced the immediate activity of the hormone by 40 per cent though the potency of the hormone was not affected if enough time was allowed for it to act. Hypoglycemia in mice and rabbits was delayed, i.e., the blood sugar fell below normal later and stayed there for longer periods, but excessively low values did not occur. The total sugar metabolized was about the same with and without the metal. No such effect was obtained when zinc and insulin were injected separately; precipitation of insulin from a zinc insulin solution with trichloracetic acid restored its initial properties. Nickel caused the same changes as zinc while cobalt and aluminum had only slight effects on the potency of the hormone (135). The hypoglycemic effect of zinc insulin may be greatly enhanced by the addition of protamin (136). Fazekas and Himwich (53) studied the effect of 0.9 per cent of zinc and aluminum on insulin in cats and dogs and found that such large amounts of metal abolished the effect, while 0.09 per cent of zinc in cats gave results similar to those observed previously by Scott and Fisher. It is interesting to notice the inactivation of penicillin by zinc, the effect of the metal resembling that of penicillinase (46).

The relationship between zinc and activation of hormones needs further clarification in view of the fact that zinc has been said to influence other hormones (15, 47, 107). The marked augmentation of the effect of hypophyseal gonadotrophic extracts as judged by production of estrus and changes in the uterus has been reported (15,

47, 97). Similar effects of copper and zinc on follicle-stimulating hormone and luteinizing hormone in the rat have been noted (54). On the contrary, the addition of zinc to equine gonadotropin decreases its activity (88). The physiological significance of these findings is not clear.

Porphyrins. It seems certain that a zinc uroporphyrin exists. Watson *et al.* have found the zinc complex of the Waldenström uroporphyrin (183) to be a constant constituent of the urine and feces in cases of intermittent acute porphyria. Urinary excretion alone seemed to be the rule in the acute, intermittent cases. In fact, the occurrence of the zinc porphyrin complex is characteristic of this type of porphyrinuria while the congenital type usually excretes the porphyrin in a free state (185–188). In these cases a zinc uroporphyrin is found in the liver. A zinc coproporphyrin has also been described (188). Zinc coproporphyrin has been found in cases of lead poisoning (113, 188), and its presence in the urine of acute rheumatic fever has been confirmed (71).

Zinc coproporphyrin has been described in bouillon cultures of *C. diphtheria* (31). The investigators were unable to state whether the zinc porphyrin was present in the bacterium or formed in the solution after disintegration. The relationship of zinc to porphyrins is not understood at present and its physiological significance is unknown.

ZINC AND ENZYMES

Reference has been made above to the examination of relationships of enzyme systems to zinc deficiency states and will not here be referred to again. Holmberg's mention of 0.13 per cent of zinc in uricase has received attention. The author's own critical evaluation of the probable metal contamination of his 'pure' preparation by the source material has been overlooked (64). Praetorius' recent work disregarding Holmberg's original conjectures as to the source of the zinc makes it virtually certain that uricase is not a zinc enzyme, thus disproving what Holmberg never claimed (124).

It has been suggested that zinc might be an active part of the kidney phosphatase molecule, but the data afford no evidence in this regard (25).

Zinc, copper, manganese, magnesium and iron have been found in kidney phosphatase (106). The presence of so many elements, none of which accumulated as the enzyme was purified, throws doubt on the significance of the observation. Roche *et al.* thought that they could activate alkaline phosphatase by the addition of zinc sulphate in concentrations of 1.10^{-5} mM. Using three different techniques for estimating phosphatase activity they found that the serum alkaline phosphatase of 97 out of 101 non-cancer bearers (96%) was activated while the enzyme in the serum of 156 out of 185 (85%) was inhibited (125). A recent report disputes these findings (17).

Warburg and Christian believe zymohexase to be a zinc complex; but the quantities of zinc present are so small that they may be impurities (184). Yudkin and Fruton have reported the activation of dehydropeptidase by zinc ions (192).

The only physiological role of zinc which has been conclusively proven to date is related to its presence as an active component of the carbonic anhydrase molecule. Because of the established correlation of enzyme activity with zinc, the properties and behavior of carbonic anhydrase will be given special attention.

That zinc is a constituent of the enzyme carbonic anhydrase was first noted by Keilin and Mann (73, 74). Leiner and Leiner (97) and Hove *et al.* (69) corroborated these findings, and Day and Franklin (35) found zinc in carbonic anhydrase extracted from plants. Keilin and Mann (73, 74) found 0.3 per cent zinc in what appears to have been a highly purified preparation of the enzyme from ox blood. Human carbonic anhydrase has never been successfully crystallized. A dithizone method was used in these analyses. Scott and Fisher (139), using analytical methods of inadequate accuracy (130), recorded the finding of approximately half that amount of zinc. They later confirmed their own results by polarographic methods (140). The discovery of the enzyme seven years previously by Meldrum and Roughton (109, 110) and by Stadie and O'Brien (149, 150) working independently was an outgrowth of the work of earlier physiologists who showed that the reaction $CO_2+H_2O\rightleftarrows H_2CO_3$ proceeded at a rate far too slow without catalysis to permit the existence of mammalian life; the reaction $H_2CO_3\rightleftarrows HCO_3^-+H^+$ is extremely rapid and requires no catalysis. The first of the above reactions is accelerated markedly by carbonic anhydrase. Zinc is an integral part of the molecule of carbonic anhydrase, for removal of the metal by hydrolysis results in inactivation of the enzyme (74); inactivation also is effected by substances which combine with zinc such as cyanide (74, 80, 111, 112, 150, 174), sulphide (74, 80, 111, 150), azide (74, 111) and BAL (189); the findings of van Goor (174) and Leiner (97) in regard to sulphide are discrepant.

Other properties of the enzyme include inhibition by carbon monoxide found by some (111, 112, 174) and disputed by others (80), and inhibition by heavy metals, observed by many (111, 129, 174), and disputed by Leiner and Leiner (97). Certain other inhibitors, widely used in attempts to demonstrate the presence and elucidate the function of carbonic anhydrase are ably discusssed by van Goor (181).

The enzyme is a protein whose low molecular weight, first suspected by Meldrum and Roughton from its behavior with ammonium sulphate (111), was later estimated to be approximately 30,000 from studies made in the ultracentrifuge (43, 122) and in the Tiselius apparatus (122, 147). Its isoelectric point is in the neighborhood of 5.6. It apparently contains no SH group (59, 142) although Bradfield felt that the enzyme in plants might (19). The enzyme is reversibly inactivated by oxidizing agents (9, 80) and permanently inactivated by heat. Its activity has been found to be greatly influenced by the concentration of various inorganic and organic substances commonly found in blood and tissues (9, 77, 90–95, 97, 99, 102–104, 141, 142, 178–180). It is felt by some (7–9, 75, 76, 78, 181) that the extreme findings of Leiner in this respect (90–95, 97, 99) require substantiation before acceptance.

Methods of Measurement. Methods of estimating carbonic anhydrase are based on measurements of acceleration of the rate of combination of carbon dioxide with or release from a substrate. In this connection it cannot be emphasized too strongly that many substances accelerate the reaction $CO_2+H_2O\rightleftarrows H_2CO_3$ (79, 80). Accordingly, it must be borne in mind that slight or moderate acceleration of the reaction by complex mixtures, such as extracts of tissues, does not necessarily indicate the presence of carbonic anhydrase itself; all methods now available measure only activity as represented by non-specific acceleration of a chemical reaction, and not the amount of the enzyme present.

The methods themselves fall into two categories: *a*) There are those which measure the rate of combination of introduced carbon dioxide with a substrate mixture at an alkaline *p*H; this may be followed colorimetrically as first formulated by Brinkman (20) and later modified by others (123, 129) or electrometrically, as proposed by Wilbur and Anderson (190). Disadvantages of these methods arise from the fact that *1*) there is no linear relation between enzyme concentration and activity (123, 174); *2*) the *p*H changes markedly and, therefore, determinations must be made at low temperature, i.e., 15° C. or below, and, *3*) indicators used in the colorimetric methods inhibit the enzyme to some extent (24, 80, 190). Clark (24) has recently criticized the electrometric methods. *b*) In the second category is manometric measurement of the rate of evolution of carbon dioxide from a bicarbonate solution upon addition of an acid buffer. The original method of Meldrum and Roughton (111) has several disadvantages and its modification by van Goor (181) involves a complicated apparatus and technique. *a*) One disadvantage of the Meldrum and Roughton method is that it uses an arbitrary unit of activity in spite of the fact that the other early workers, Stadie and O'Brien (150) and Kiese and Hastings (80) calculated activity by means of velocity constants; recently, however, Mitchell *et al.* (116) applied a simple method for calculation of velocity constants to the technique and so supplanted the original arbitrary unit with one of functional significance; Clark (24) has contributed further discussion in this regard. *b*) Another disadvantage of the original method lay in the fact that measurements had to be made at temperatures of between 0° and 15° C., usually 0°. It is well known that the use of low temperatures exaggerates the apparent activity of the enzyme (74, 103, 111, 127). For three years a method has been in use which uses the temperature 38° C. (1); this has been found satisfactory for clinical studies. The method was discussed with Roughton and within a very short time he was able to corroborate the fact that higher temperatures than 15° C. could be used; he also showed that acceleration of carbon dioxide output by the enzyme at 38° C. is only approximately 5 per cent of that obtained when activities of the same preparations are studied at 0° C. (117). Moreover, extreme cold inactivates the enzyme (78, 80, 128). *c*) Another disadvantage of the original method was the violent shaking required; this may inactivate the enzyme (89, 93, 99, 128, 129). However, Leiner (89, 93, 99) and Stadie *et al.* (151) showed that the Warburg apparatus could be used, a finding which was corroborated by Altschule and Lewis (1) and later by Krebs and Roughton (84). *d*) The original method yielded data which showed that there was no linear correlation between enzyme concentration and enzyme activity when blood was used although such a correlation was present when purified enzyme was used, but only in very dilute solution (87, 111). A method, using extrapolation, has been devised for surmounting this difficulty in measuring carbonic anhydrase activity in blood (1). *e*) All manometric methods use substrate and buffer mixtures, and *p*H ranges which do not permit of estimation of activity of the enzyme *in vivo*. The recently published work of Roughton and Booth (128) appears to represent the application of elegant refinement in calculation to a method of moderate precision and of low validity for mammalian physiology.

The relation between zinc and carbonic anhydrase in unpurified biological material was first studied by Leiner and Leiner (89). These authors using methods of

doubtful validity found a relation between zinc concentration in various organs and the amount of carbonic anhydrase extractable from the latter. These data are difficult to accept because of inadequacy of the methods used. Later, Leiner (96) and Scott and Mendive (142) stated explicitly that no close parallelism existed between zinc content and enzyme activity. This must certainly be true of tissue extracts for in them zinc is present in many forms. However, in a more recent study of carbonic anhydrase and zinc in human blood methods of high accuracy were used (172). It was shown that a close parallelism exists between carbonic anhydrase activity and zinc content in erythrocytes, normal adult human blood and also in blood in a wide variety of diseases. So close was the correlation between the two that it was suggested that zinc be used as a measure of carbonic anhydrase *content* of erythrocytes, a value that otherwise is not ascertainable. The enzyme is present in large amounts in mammalian blood (1, 2, 5, 73, 86, 87, 100, 111, 174–177); it is all within erythrocytes, for no activity is found in leukocytes or in plasma (1, 181); Lambie reported a very small amount of activity in human plasma (87) and Booth found a little in pig serum (18). The best evidence indicates that red cell carbonic anhydrase content of erythrocytes, judging from their zinc content is probably one per cent of the hemoglobin content. The amount of carbonic anhydrase in dog blood is somewhat less, judging from the smaller amount of zinc found in the red blood cells of that species.

The blood of mammalian embryos or newborn exhibits low carbonic anhydrase activity, as was first noted by Meldrum and Roughton (111), van Goor (174) and Lambie (87) and later studied extensively in infants by Stevenson (153); the blood of premature infants has even less activity. The methods used by all of these authors exaggerated the deficiency of the enzyme in the blood; recent studies (1) show a lesser degree of lowering of activity in the blood of newborn infants so that the carbonic anhydrase activity per ml. of blood, enhanced somewhat by the polycythemia common at this stage, is only about 50 per cent less than that of the normal adult, with the blood of premature infants at a somewhat lower level (3). Studies on the zinc content of blood in newborn infants are not available.

In addition to the fragmentary studies of Wille (191) and of Hodgson (63) in anemia, there is a considerable body of data published by Lambie (86, 87) who used the original Meldrum-Roughton technique. Studies on bleeding in manmmals have also been reported (87, 178). More recently, studies by a better method corroborated Lambie's (86, 87) conclusions as to the parallel decrease of carbonic anhydrase activity and blood hematocrit cell percentage and hemoglobin concentrations in patients with hemorrhage (100, 172); in addition, it was shown that the blood of patients with anemia associated with nutritional iron deficiency, uremia and infection exhibit similar findings. In pernicious anemia, on the other hand, the blood carbonic anhydrase activity is in or near the normal range in spite of very low hematocrit cell percentages and hemoglobin concentrations (100, 172); this phenomenon is not due solely to large cell size, for the mean corpuscular carbonic anhydrase activity is increased several times more than the increased cell size and elevated mean corpuscular hemoglobin values. In patients with anemia associated with liver disease or with leukemia the carbonic anhydrase may be reduced in proportion to the hematocrit cell percentages and hemoglobin concentrations, or may be disproportionately high,

though not necessarily as much as in pernicious anemia. The statement by Lambie (87) that hemolytic anemias are associated with disproportionately high blood carbonic anhydrase activity is not borne out by his own data, for only one of his eight cases showed this phenomenon; this disproportion was not found in a more recent study either (100). The significance of change, or lack of change in carbonic anhydrase activity in various types of anemia cannot be stated but it is of interest that in pernicious anemia exertional dyspnea is absent or notably mild in spite of low hemoglobin concentrations. At any rate, it is worthy of note that whatever the level of carbonic anhydrase activity in the blood, the concentration of zinc in the erythrocytes is at all times proportional to the activity of the enzyme in anemia (172).

In patients with polycythemia vera, the blood carbonic anhydrase activity is increased in proportion to the hematocrit cell percentage and the hemoglobin concentration (100); a similar situation obtains in patients with polycythemia secondary to chronic pulmonary disease (2). Here again a close parallelism between enzyme activity and erythrocyte zinc concentration is found (172).

Discussion of the carbonic anhydrase content of tissues is beyond the scope of this review; this material has been covered thoroughly in van Goor's excellent review (181). It should be re-emphasized, however, that since zinc exists in forms other than or in addition to carbonic anhydrase in tissues, the use of zinc levels for estimating enzyme content or supporting measurements of enzyme activity is not valid in biological material other than blood. The confused state of the literature regarding the presence and function of carbonic anhydrase in tissues, ably delineated by van Goor (181) is understandable; precise knowledge regarding the enzyme must wait on the isolation and intensive study of the enzyme, the formulation of better methods for estimating activity, and the development of specific chemical methods for measuring the amounts of enzymes present in various tissues.

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THIAMINASE, THE CHASTEK-PARALYSIS FACTOR

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THE INCLUSION of raw fish in diets fed to foxes on fox ranches has resulted in severe outbreaks of a paralyzing disease which, left untreated, is fatal. R. G. Green first noted this condition in 1932 on the fox ranch of J. S. Chastek of Glencoe, Minnesota. Four years later he published a description of the disease which he called Chastek paralysis (19). Ultimately it was found that Chastek paralysis is due to a thiamine deficiency induced by a heat-labile factor in certain species of fishes which renders the normal dietary thiamine unavailable to the fox. Shortly the Chastek-paralysis factor was shown to be an enzyme which splits thiamine into its pyrimidine and thiazole components, and thus persisted the name thiaminase (56) although this name had been suggested earlier by Bonner and Buchman (8) for an enzyme in pea roots capable of synthesizing thiamine. Three reviews (40a, 41, 1) have appeared concerning the nutritional aspects of thiaminase. Considerable work has been done, however, on the nature and distribution of this enzyme, as well as on the deficiency it produces. This review proposes to cover most of the literature available at present.

CHASTEK PARALYSIS

Symptoms. In the period from 1932 to 1936 Green studied 4 acute outbreaks of a hitherto undescribed disease of foxes. About one month after the introduction of raw fish to the diet of foxes on the ranch of J. S. Chastek, Glencoe, Minnesota, 34 per cent of these animals rapidly developed a spastic paralysis which resulted in death in 1 to 4 days after the onset of symptoms. The fish employed were frozen immediately after catching and thawed only at the time of feeding, so that no spoilage could have occurred. In the very beginning it was shown that Chastek paralysis was neither food poisoning nor was it transmissible from fox to fox as an epizootic disease. These occurrences of Chastek paralysis, moreover, were on widely separated ranches, all of which fed fresh fish. In all cases the disease stopped once the fish was removed from the diet (19).

Once paralysis set in, the course of the disease was rapid and the symptoms became more characteristic (19, 28). Several weeks after the feeding of fresh fish was begun, the foxes began to refuse food. In the interval, however, no harmful effects were evident from the change in diet. The anorexia increased during the following week until some of the foxes were leaving all of their ration. Stiffness of gait, the first sign of actual disease, occurred after a week or two of anorexia. Within 12 to 36 hours these foxes showed extensive spastic paralysis so that they could not rise,

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or stand if they were placed on their feet. The animal displayed a general, abnormal sensitivity to pain and remained conscious although it was almost completely paralyzed. Convulsions often occurred, and the animal usually died within 12 hours after total paralysis of the limbs had set in.

Etiology. Green (20) announced that Chastek paralysis was due to a vitamin B deficiency caused by fresh whole fish when it makes up 10 per cent or more of the foxes' diet. Below is a diet which produced *no* outbreak of Chastek paralysis in January 1935 (21).

Cereal	10.0%	Fresh fish	7.0%
Fresh vegetables	10.0	Cod liver oil	0.1
Liver	8.0	Dry milk	2.0
Horse meat	50.0	Water	12.9

In January of the following year, however, when the diet was modified to include 18.0 per cent raw fish, 2.0 per cent eggs and 2.0 per cent liver, a severe outbreak of Chastek paralysis occurred. At that time the vitamin B deficiency was attributed to the increased demand for the vitamin made on the fox by the metabolism of the fish oils together with the comparatively poor source of vitamin B that fish provides. In the above disease-producing diet the amount of liver and eggs fed was considered not enough to prevent the avitaminosis.

An inherent objection to this hypothesis was that cooked fish could be fed in large amounts with impunity. Since Chastek paralysis was a neurological manifestation it was assumed at first that thiamine was the B-factor involved. Cooking is very likely to decrease, rather than increase, the thiamine content of the fish. Lunde (38) showed that the food items in diets which produced Chastek paralysis contained essentially the same amount of thiamine as diets which produced no symptoms of pathology. Coombes (10) claimed that Chastek paralysis was not caused by a thiamine deficiency because he was able neither to produce the disease in animals fed a thiamine-deficient diet, nor to cure the fish-diet disease by feeding thiamine in large amounts. He attributed the paralysis to specific heat labile toxins occurring in fresh or partially decayed fish. Investigators in Norway, however, were able to produce a condition, the symptoms of which very much resembled Chastek paralysis, by feeding foxes a diet deficient in thiamine (16, 36). Thiamine injected into these animals resulted in the disappearance of paralysis. What appears to have been a typical outbreak of Chastek paralysis in Sweden was checked by administering thiamine to the sick animals and reinforcing the diet with dried yeast (9). Although the presence of fish in the diet was noted, the occurrence of the thiamine deficiency was not linked with the feeding of fish.

It soon became apparent that the thiamine deficiency resulting from feeding raw fish occurred, not because the thiamine content of the diet was too low, but rather because the fish in some way was making the dietary thiamine unavailable to the fox.

Pathology. In Chastek paralysis the diagnostic lesions at necropsy are found in the brain when examined microscopically. These lesions were noted in the early studies (19, 21) and were described later in much greater detail (17). Characteristic changes, however, are often found in other organs.

In gross examination the appearance of the heart may suggest Chastek paralysis. It shows massive degeneration with cloudy swelling, in some cases so severe, that the authors use the term 'cooked' to describe it. In about one-half of the animals hemorrhages are present. Microscopic examination reveals large nucleoli and focal necrosis in myocardial fibers, proliferation of connective tissue arising from the vascular tree, and hemorrhages. This combination of changes, although one or more is frequently absent, is indicative of Chastek paralysis.

The liver usually shows degeneration but its gross appearance is quite variable, at times fatty, or of a dark mahogany color. Most of the viscera other than the heart and liver do not show any effects of Chastek paralysis.

Most significant and diagnostic, however, are the lesions which occur in the brain and spinal cord of animals with this disease. Sections of the brain and spinal cord reveal small spot-like hemorrhages which can usually be seen grossly. Microscopic examination reveals that typically the lesions are bilaterally symmetrical and do not extend into the white substance. The changes appear to be vascular; small blood vessels have undergone irregular dilatation and vast proliferation of their endothelium. The lesions involve only certain of the paraventricular nuclei and also are commonly found in the inferior olive and in the cortex of the splenial gyrus. Despite the fact that these changes are vascular they are never encountered in the white substance.

The brain lesions of Wernicke's disease due to chronic alcoholism in man have been shown by Alexander (3) to be identical with those induced experimentally in pigeons by feeding a thiamine-deficient diet with an ample supply of the other vitamins. Additional support for the theory that Chastek paralysis is caused by a thiamine deficiency was furnished by the fact that the distribution and histological appearance of the lesions in this disease were identical with those found by Alexander in his study of Wernicke's disease (27, 4).

Nursing Fox Pups. The pathological findings described above are not present in nursing fox pups which die from Chastek paralysis. In these, the disease takes an acute form; Green (22) has described outbreaks of Chastek paralysis in fox pups in which one-third of the nursing pups on a ranch have succumbed in a period of 3 days. The severity of the disease in pups has been attributed to the absence of thiamine in the mother's milk, since lactating females with Chastek paralysis would tend to conserve thiamine for their own use (28). It has been shown that lactating animals with sub-acute vitamin deficiencies induce acute deficiencies in their young, retaining the vitamin for themselves (42). Thiamine analyses, however, have not been performed on the milk of lactating foxes with Chastek paralysis. The absence of the neurological pathology in the fox pups has been explained on the possibility that Chastek paralysis in its acute form does not give a chance for the vascular changes to occur before it interferes with a vital function. Under these circumstances it appears that the vascular changes may in themselves not be responsible for the death of the animal. Treatment of the affected fox puppies with thiamine effected a rapid and complete cure.

Therapy. By the end of 1940 the cumulative evidence pointed to Chastek paralysis as definitely a thiamine deficiency (27). Possible seasonal occurrence of the

disease had been explained by the fox farmers' habit of feeding raw fish during the winter months when the foxes' sources of protein become restricted (23). Shortly after crystalline thiamine chloride hydrochloride was available, Green, Carlson and Evans (25) showed that a diet with 20 per cent raw carp which produced Chastek paralysis in controls would not produce the disease at all when it was supplemented by 25 mg. thiamine per day. Moreover, cures of Chastek paralysis were effected by the subcutaneous injection of 20 mg. thiamine although, as was admitted by the authors, the supply of foxes with the disease was too limited to allow the simultaneous use of untreated controls.

The objections by Coombes (10) to the theory that the etiological factor was a lack of thiamine were withdrawn (11). Despite earlier unsuccessful attempts by Coombes (10), Hodson and Smith (30) were able to produce the clinical symptoms of Chastek paralysis in a group of foxes fed a diet deficient in thiamine but containing no raw fish. Brain lesions typical of Chastek paralysis were not, however, found in the only 2 foxes of this group subjected to post-mortem examination. Simultaneous cases of Chastek paralysis for comparison of behavior were obtained by including raw smelt in the otherwise adequate diet of a second group of foxes. Only thiamine, and not riboflavin, pyridoxine or calcium pantothenate, would cure both the deficiency syndrome and the Chastek paralysis. Previously it had been shown that nicotinic acid was not therapeutic (25).

Three possibilities of how fish are able to cause thiamine deficiency presented themselves: "First, fish may somehow increase the animals' need for vitamin B_1 by a metabolic effect; second, fish may prevent absorption of the vitamin; and third, fish may bring about an inactivation or destruction of the vitamin in the food" (25).

INACTIVATION OF VITAMIN B_1

Shortly after the work of Green, Carlson and Evans (25) proving that Chastek paralysis is a manifestation of B_1 avitaminosis many workers took up the investigation to find out how the raw fish rendered the thiamine unavailable. Spitzer *et al.* (55) showed that typical polyneuritis can be made to occur in chicks by feeding 25 per cent whole raw carp in an otherwise satisfactory basal ration. Especially significant are the results of their *in vitro* experiments in which they incubated raw or cooked carp entrails with thiamine chloride hydrochloride and then attempted to recover the added vitamin B_1 by means of the thiochrome method. In this way they showed that the destruction of the thiamine occurs within the food mixture itself and that the inactivation of the vitamin was proportional, more or less, to the length of time it was in contact with the raw carp entrails. In addition, they confirmed the heat lability of the factor and indicated that the raw entrails were more potent than the whole carp in producing chick polyneuritis.

At approximately the same time, Green (24) demonstrated that raw fish could be fed with impunity provided the vitamin B_1 of the diet did not come in contact with it in the diet mixture. The fish and a balanced diet were fed on alternate days. Carp fillets were shown by Green, Carlson and Evans (26) not to produce Chastek paralysis. On the other hand, the disease was readily produced by feeding the external structures (scales, fins, skeletons and heads) or the viscera of carp in the diet. The feeding of 10 mg. thiamine per day was sufficient to prevent Chastek paralysis.

Woolley (59) was able to obtain extracts of high activity to inactivate thiamine *in vitro* by extracting fresh carp tissue with 10 per cent sodium chloride solution. Aqueous extracts of carp, however, had only 25 per cent of the activity of whole carp suspensions. These, like the earlier investigations (55, 26), pointed to the Chastek paralysis factor as being either an enzyme which destroyed thiamine or a protein which combined with whole thiamine to render it unavailable; an analagous situation has been found in the combination of avidin with biotin (14). Additional light was thrown on this by Deutsch and Ott (13) who demonstrated that the thiamine of non-viable yeast is destroyed by keeping it in contact with raw smelt and that the thiamine so destroyed cannot be recovered by mild acid hydrolysis, a treatment which releases biotin from the avidin-biotin complex. Yet, like avidin, the Chastek-paralysis factor was found unable to penetrate the living yeast cell, for the thiamine

TABLE 1.[1] DISTRIBUTION OF THIAMINE-DESTROYING PRINCIPLE IN CARP TISSUES

TISSUE	WEIGHT	ACTIVITY UNITS	TISSUE	WEIGHT	ACTIVITY UNITS
	gm.	*per gm.*		*gm.*	*per gm.*
Spleen	7.8	25.00	Kidney	17.8	1.34
	2.5	6.00	Blood		1.23
	8.2	21.6			0.80
Liver and pancreas	95.1	2.5	Heart	4.1	0.33
	53.9	1.90	Testes	392	0.25
	34.7	1.48	Brain	2.3	0.21
Gastrointestine	44.4	2.5	Mucous	21.2	0.04
	31.3	8.72		3.7	0.14
	23.7	3.54	Gall bladder and bile	8.4	0.11
Gills	52.1	2.5	Eyes	7.8	0.06
	34.0	4.34	Ovaries	9.3	0.62
	15.2	2.16	Swim bladder		0
			Muscle		0

[1] SEALOCK, LIVERMORE AND EVANS (52).

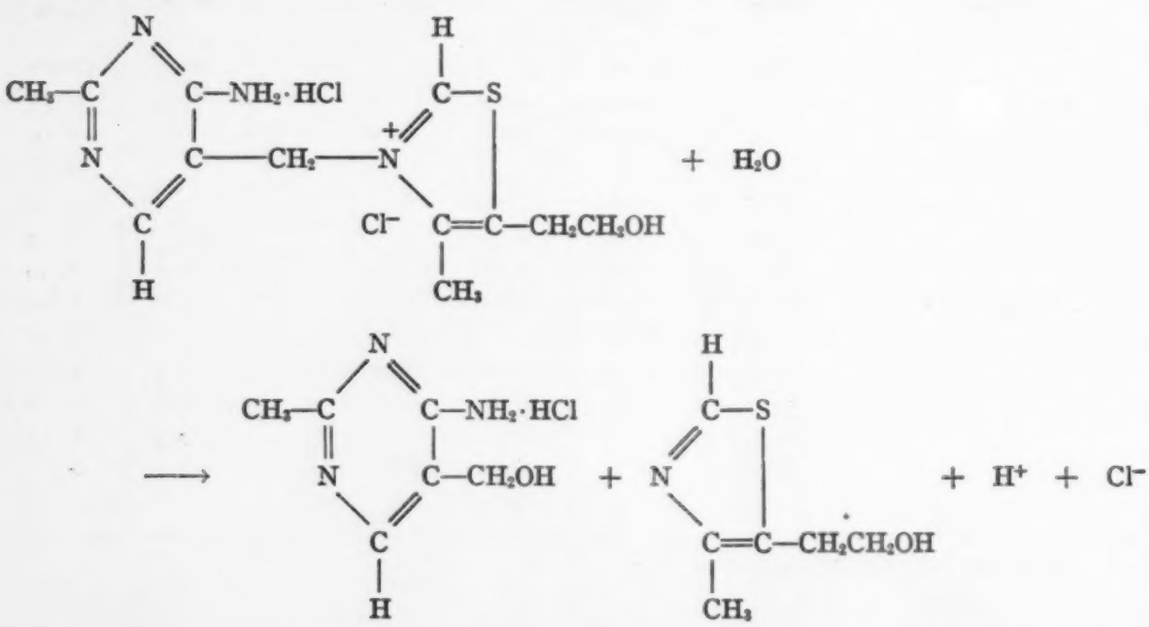
content of viable yeast was not lowered significantly when the yeast was mixed with raw smelt.

The properties of the *in vitro* inactivation of thiamine were studied by Sealock, Livermore and Evans (52) in some detail. Using Woolley's technique for extracting the factor which destroyed thiamine, they assayed various organs and structures of the carp. Their results are summarized in table 1 from which it can be seen that the greatest activity resides in the spleen of the carp and that the muscle of the fish is inactive. One unit of activity was defined as that amount of activity which at *p*H 7.4, 37.5° C., at a thiamine concentration of 0.5 micromoles per ml., will cause the disappearance of 1 micromole of thiamine. These authors prepared a stable desiccated powder from acetone-extracted minced carp viscera from which they were able to extract the thiamine-inactivating principle with 10 per cent NaCl solution. With this preparation they found that the maximum disappearance of thiamine occurred at *p*H 9.1, with suitable corrections for the thiamine lost by alkaline hydrolysis. The activity of the preparation was greatest at 60° C.; raising the tempera-

ture of the extract to 100° for 30 minutes completely inactivated the principle. The disappearance of the thiamine was approximately proportional to the amount of the extract used and fitted more or less the kinetics of a first-order reaction. These properties were advanced by the authors as all evidence in favor of the *enzymatic* destruction of thiamine.

ENZYMATIC DESTRUCTION OF THIAMINE

Cleavage of Thiamine. Although the kinetic studies of Sealock *et al.* (52) suggested that the inactivation of thiamine by carp tissue was an enzymatic reaction, the final proof of this hypothesis was achieved by Krampitz and Woolley (35) who isolated the products, 4-methyl-5-hydroxyethylthiazole and 2-methyl-4-amino-5-hydroxymethylpyrimidine, of the thiamine cleavage from a thiamine-free mixture of carp tissue incubated with thiamine. The reaction, therefore, was a hydrolytic cleavage of the thiamine molecule between the pyrimidine and thiazole rings as shown below.



It should be noted that this hydrolytic cleavage sets free a proton from the water molecule; the tertiary nitrogen of the thiazole moiety will, in acid solution, form a salt with this proton in covalent linkage, the nitrogen becoming pentavalent. The release of this proton, however, would serve to lower the *p*H as the reaction progressed.

Krampitz and Woolley (35) have suggested that the destruction of thiamine involves more than one step. *Mucor ramannianus*, a mould which requires merely the thiazole portion to fulfill its thiamine requirements, was used satisfactorily to assay the dialysates of thiamine-free enzyme mixtures made with thiamine incubated with either carp tissue or NaCl extracts of carp tissue. On the other hand, *Endomyces vernalis*, a yeast which requires merely the pyrimidine portion to fulfill its thiamine requirements, showed that only a small fraction of the activity of the original thiamine remained in the dialysates of thiamine-free enzyme mixtures made by incubating thiamine with NaCl *extracts* of carp tissue. When similar mixtures

made with carp *tissue*, rather than extracts, were assayed with *Endomyces* all the activity of the original thiamine was accounted for. In all cases a chemical method was used to assay for whole thiamine. Treatment with alkali of the thiamine-free reaction mixture of extract incubated with thiamine restored full activity in tests with *Endomyces*. Standing for long periods also increased the ability of these mixtures to promote the growth of the yeast. On this evidence the authors postulated a two-step reaction. The first, carried out in extracts of carp tissue, would be to split the thiamine into thiazole and a pyrimidine derivative with but slight activity for *Endomyces*. The second step, carried out by a substance present in the carp tissue but not in the extract, would serve to convert this pyrimidine derivative into 2-methyl-4-amino-5-hydroxymethylpyrimidine. This hypothesis has been investigated further by Hennessy (29) but as yet his results are not available.

In their investigations Krampitz and Woolley (35) noted that most of the thiamine-destroying potency of carp extract was lost upon dialysis against distilled water. The activity of the extract was fully restored, however, upon addition of the dialysate which in itself was inactive. The factor in the dialysate, moreover, was heat stable, whereas that in the undialyzable fraction was heat labile.

The rate of destruction of thiamine by carp extract was increased by raising the temperature and the *p*H. Temperatures over 37° C. were not tested but the temperature coefficient over the range 0 to 37° was small. Thiamine destruction proceeded at *p*H 8 almost twice as fast as at *p*H 5. At *p*H 1 the reaction went at about half the rate at *p*H 5 and the authors believe that this is due in part to the destruction of the enzyme at *p*H 1. Values above *p*H 8 were not tested. The rate of destruction of thiamine by a given amount of carp extract was determined. In one example, the cessation of thiamine destruction after the first 30 minutes, when about three-fifths of the added thiamine was split, was shown by the authors not to be due to an inhibitory action by the breakdown products. Krampitz and Woolley (35), however, give no data concerning initial *p*H or control of *p*H in any measurement of enzyme activity other than that done to find the effect of *p*H. As such, it is difficult to evaluate their results in some experiments.

Assay of Thiamine. At this point a few words must be said concerning the chemical determination of thiamine. In the investigations of the behavior and characteristics of thiaminase, the work of Sealock *et al.* (52), Krampitz and Woolley (35), Lieck and Ågren (37), and others to be mentioned below, the method for the determination of thiamine was a modification of that described by Melnick and Field (39). This method involves the adsorption of the vitamin on zeolite, its elution with KCl, and treatment of the eluate with diazotised *p*-aminoacetophenone (the Prebluda-McCollum (43) reaction). The resulting pigment is extracted with xylene and measured colorimetrically. In many of the studies on thiaminase, however, the zeolite adsorption was omitted. Krampitz and Woolley (35), however, adsorbed the thiamine on Super Filtrol and determined the thiamine in the adsorbates according to the method of Emmett, Peacock and Brown (15) which again is essentially based upon the Prebluda-McCollum reaction (43).

In their investigations, Lieck and Ågren (37) were rarely able to recover entirely thiamine added to fish extract the protein of which was then precipitated by trichlo-

roacetic acid; at times their loss was as much as 50 per cent. Except for their omission of the adsorption step, their procedure was that of Sealock *et al.* (52) who, although they did not report the loss of thiamine in controls, were able to assay a given extract in terms of units of thiaminase with an average error not greater than ±5 per cent. Ågren (64) later found that, among other agents, cysteine and ascorbic acid can inhibit the formation of color in the modified Melnick-Field method. Whether this is due to the omission of the adsorption step is not certain. Sealock and Goodland (49), who also reported interference in color development by cysteine, presumably adsorbed the thiamine on to zeolite. Jacobsohn and Azevedo (32) have found that extraction of fresh gold fish (*Carassius*) with M/15 phosphate (*p*H 6.9) yields an extract which does not lose thiamine when the protein is precipitated; they believe that the thiamine is lost by adsorption on to the precipitated proteins rather than by a non-specific interference with the Melnick-Field color. But their recovery of thiamine may in fact be due to the use of the thiochrome procedure for the assay. Ågren (64) noted that those factors interfering with color development in the modified Melnick-Field method do not interfere in the thiochrome method. The results of Krampitz and Woolley (35) show that they were able to recover 96 per cent of the thiamine added to boiled extracts. Their direct adsorption on to Super Filtrol at *p*H 2–3 avoided protein precipitation with trichloroacetic acid. In all their calculations these authors imply that their recovery was 100 per cent.

It will be noted below that some investigators have used a thiochrome assay method. In view of the foregoing it would appear that this method is preferable to the use of the Melnick-Field method, especially when the latter method is modified to exclude the adsorption procedure. Sealock and Livermore (50) have suggested a manometric method for the measurement of thiaminase activity. Since the cleavage of one molecule of thiamine is accompanied by the release of a proton, these authors have measured the liberation of extra carbon dioxide in a bicarbonate buffer. Comparison with chemical determinations of thiamine lost, showed that, as developed, the method gave results that were not at all reproducible.

Thiaminase

Purification. In his first efforts Ågren (61) succeeded in purifying thiaminase about ten times by isoelectric precipitation between *p*H 5.0 and *p*H 5.3. Since this method did not always give reproducible results, the following procedure was adopted (62): Finely ground fish (tench, *Tinca vulgaris*, or ide, *Leuciscus idbarus*) viscera were shaken for 1 hour at 0° C. with an equal volume of water, and then centrifuged for 1 hour at 3,000 r.p.m. in the cold. The precipitate was discarded and the centrifugate acidified to *p*H 5.7 and recentrifuged. The precipitate was discarded and solid ammonium sulphate was added until the centrifugate was 0.6 saturated. With Hyflow Super Cel the precipitate was filtered off by vacuum and discarded. The clear filtrate was then completely saturated with ammonium sulphate and the resulting precipitate spun off. Dissolved in water, this precipitate gave a thiaminase preparation which showed a 10-fold increase in activity over the original water extract. Cataphoretic separation and short dialysis of the partially purified enzyme resulted in still another 10-fold increase in purity (63). The resulting solution was clear and

colorless, and was 100 times as active as the original aqueous extract of the fish viscera. The activity of most of these preparations was not stable even when the material was stored at −15° C. Units of thiaminase activity were those employed by Sealock *et al.* (52) and were measured in terms of mg. protein nitrogen.

Characteristics. Inactivation of carp thiaminase by dialysis was also accomplished by Lieck and Ågren (37) in their studies on the distribution of the enzyme in various species of fishes. The activity of the enzyme prepared by extracting the fish viscera with water was estimated by the method of Sealock *et al.* (52). Lieck and Ågren (37) showed the inactivation of thiamine by carp extract was not an oxidative process since neither was oxygen uptake noted in manometric experiments nor was methylene blue reduced in experiments carried out in Thunberg tubes. Evidence for reductive inactivation was presented by Ågren (61) when he showed that the activity of dialyzed, completely inactive enzyme was restored by the addition of glutathione. The enzyme, freed from accompanying catalase by isoelectric precipitation, was then partially inactivated by hydrogen peroxide and again reactivated by glutathione but in this case the glutathione concentration was high enough to interfere with color development in the Melnick-Field method. It is doubtful whether the thiamine is reduced, since sodium hyposulphite does not interfere with the assay except when in high molar ratio to thiamine.

Whether glutathione, in activating dialyzed thiaminase, is acting as a coenzyme or reducing the enzyme to an active state has not been ascertained. The effect that glutathione exerts by activating these preparations is very likely not due to interference with color development for two reasons: *1*) the concentration of glutathione is below the minimum which interferes and *2*) addition of glutathione in the same concentration to an extract prepared from a fish which does not contain thiaminase does not cause that extract to show thiamine destruction under the experimental conditions. Whether glutathione, or any reducing substance, is, indeed, the true coenzyme cannot be said for certain. Ågren (61) found that extracts of fish which did not contain thiaminase could not be induced to destroy thiamine upon the addition of the dialyzable factor from extracts of fish which did contain the enzyme. No one has observed, however, whether the dialyzable factor is present in fish, extracts of which do not destroy thiamine.

Recently Sealock and Livermore (51) have restored the activity of dialyzed thiaminase with manganous chloride (2×10^{-4}M). So far, the data have not been given completely but cobaltous ions also were effective.

Bhagvat and Devi (7) used chloroform-water mixtures to extract thiaminase from carp tissue. They confirmed the heat stability of the co-factor and the comparative insensitivity of the velocity of the enzyme reaction to hydrogen ion concentration between *p*H 4 and *p*H 7. Extracts of the viscera destroyed 100 per cent of added thiamine (25 γ) in one hour at *p*H 5.6. The thiochrome method was used for estimating thiamine.

The study of the inhibition of thiaminase is also complicated by the agents tested interfering with the development of color in the Melnick-Field method. Sealock and Goodland (48) found that carp thiaminase could be markedly inhibited by cupric, zinc and ferric ions. Cyanide, fluoride, iodoacetate, sulphydryl and sulphite

were not as effective. Such common agents as mercuric and silver ions, potassium ferricyanide, hydroxylamine, hydrogen sulphide, cysteine, iodine and sodium sulphite interfered with color development (49). Use of the thiochrome method, however, has shown that in low concentrations mercuric ion will not inhibit goldfish thiaminase (34). Neither morphine nor acetylcholine seemed to have any effect on this enzyme (33, 34).

The thiaminase activity of carp can be competitively inhibited by o-aminobenzyl-4-methylthiazolium chloride, which bears a structure similar to thiamine. Substitution of the amino group of this compound by a nitro group abolished its capacity to inhibit the enzyme (48). If the amino group is in the *para-* position in this compound, no inhibition occurs; m-aminobenzyl-4-methylthiazolium chloride increases the velocity of thiaminase action. Varying the position of the methyl group in these compounds has no effect (51). Sealock and Davis (47) showed that *meta-* substituted aromatic amines, in this case m-nitroaniline, combine with the pyrimidine derivative resulting from the thiamine split thereby increasing the velocity of the cleavage. This would thus serve to explain the enhancing effect of m-aminobenzyl-4-methylthiazolium chloride. The inhibition of thiaminase produced by β-aminoethyl-4-methyl-thiazolium chloride was also greatly reduced upon substitution of the amino group by a phthalimido group (48). Since oxythiamine (deaminated thiamine) also inhibits thiaminase (54) it is difficult to assess the value of the amino group in the substrate for the formation of the enzyme-substrate complex.

The thiaminase of carp spleen has been shown by Beloff and Stern (6) to inhibit yeast cocarboxylase activity, probably by actual destruction of the cocarboxylase (thiamine diphosphate).

DISTRIBUTION OF THIAMINASES

The investigations of the occurrence of thiaminase in different species of fishes and in other organisms has been carried out by the feeding of the fish to be tested in order to see whether Chastek paralysis could be produced, and also by the much more rapid *in vitro* method of determining the thiamine destroyed upon incubation of the vitamin with extracts or breis of the fish to be tested. In any case it is necessary that the animal tested be identified accurately. For that reason much of the early work on the distribution of thiaminase must be discounted. Common names, especially those of fishes, will often change radically from one small locality to another. Conclusive results will be reached if the whole animal or its viscera is used for the study because most of the thiaminase activity resides in the viscera (cf. table 1).

Deutsch and Hasler (12), using an *in vitro* method on whole fish or fish viscera, examined 31 species of fish caught in the Great Lakes region of the United States for the presence of thiaminase. Fifteen of these fresh-water forms were found to contain the enzyme. All 4 species of the carp family (Cyprinidae) that they examined contained the enzyme. Of 9 marine forms examined, none contained thiaminase but in the case of the cod (*Gadus morrhua*) and the haddock (*Melanogrammus aeglefinus*) the fish had been previously eviscerated. To this list of marine teleosts Yudkin (60) added 3 more, the viscera of which did not contain the enzyme. Lieck and Ågren (37) examined 30 species of fish caught in Swedish lakes, the Baltic Sea, and the Kattegat. Altogether 10 of the 30 fish examined contained the enzyme. Of the 10

species of Cyprinidae examined, 9 contained thiaminase. The gar-fish (*Belone acus*) caught in the Kattegat, thus a salt-water form, also contained thiaminase. Later, using *Phycomyces blakesleeanus* to assay thiamine, Wiken and Ågren (57) showed that the cod (*Gadus callarias*) and the asp (*Aspius aspius*) contained small amounts of thiaminase. The slight differences that were noted, however, do not appear to be significatn.

Wolf (58) has described a disease of trout caused by diets containing raw fish. Examination of the central nervous system of diseased fish, moreover, showed that the histopathology of the condition was identical with that of Chastek paralysis in foxes and of Wernicke's disease in man (4). Fish with fish-diet disease exhibit loss of balance causing the fish to lie on their sides on the bottom of the container. When disturbed the fish exhibit great excitability so that they dash about violently. These side swimmers weaken, lose their appetite and eventually die. Administration of vitamin B_1 cures the condition. Cooked fish will not injure the trout in any manner. Wolf was able to produce this disease by feeding trout Atlantic herring (*Clupea harengus*), a salt-water form, and buckeye shiners (*Notropus atherinoides*), a fresh-water form. That herring contains thiaminase was confirmed by the work of Smith and Proutt (53) who were able to produce a thiamine deficiency in cats on a diet containing Atlantic herring. Lieck and Ågren (37), on the other hand, did not find any thiaminase in *Clupea harengus* caught in the Baltic Sea or in the Kattegat.

Clams have also been shown to contain thiaminase (Woolley, cited in Melnick, Hochberg, & Oser, 40) but the oyster has none. Species names were not given in this study but they were likely to have been *Venus mercenaria*, *Mya arenaria* and *Ostrea virginacea*. In Portugal, thiaminase has been found in the shrimp, *Penaeus caramote*, and the clam, *Tellina*, but not in the cockle, *Cardium edule*, or the crab, *Portunus marmoreus* (32). All these species are marine, but other than these slight investigations no thorough study has been made on the distribution of the enzyme among marine invertebrates.

Azevedo (5) has found no evidence for thiaminase in tortoises (*Clemmys leprosa*) or frogs (*Rana esculenta*). Actually, the experiments with *Clemmys* were equivocal.

The fresh-water mussel, *Lamellidens marginalis*, contains thiaminase with some different characteristics from that in carp. The carp enzyme possesses a *p*H optimum near *p*H 9 (52) whereas the destruction of thiamine in aqueous extracts of *Lammellidens* shows peaks of activity at two hydrogen ion concentrations, *p*H 3.6 and *p*H 6.5 (45). Evidence that, indeed, two thiaminases are present is brought forth on the fact that M/100 iodoacetic acid inhibits thiamine destruction at *p*H 3.6 but leaves thiaminase activity at *p*H 6.5 unimpaired. On dialysis the extract does not lose any activity to destroy thiamine at *p*H 3.6 but loses 50 per cent of the thiaminase activity at *p*H 6.5. Both enzymes were inhibited by M/1000 copper sulphate and activated by manganese sulphate. No additional fresh-water invertebrates have been examined.

It is notable that thiaminase occurs to a great extent in the Cyprinidae only because that is a pattern that has been at all consistent. Other than the Atlantic herring (*Clupea harengus*) and the gar-fish (*Belone acus*) no marine teleosts have been shown to contain thiaminase. Although the early reports of Green, Carlson and Evans (26) claimed that the Atlantic whiting (*Merluccius bilinearis* ?) was responsible for the development of Chastek paralysis in foxes, subsequent *in vitro*

studies (12, 60) have not demonstrated the presence of thiaminase in this form. Needless to say, no conclusions on a phylogenetic basis can be examined about the distribution of thiaminase until more data in diverse animals are available. Present evidence that two, or possibly three, thiaminases may exist also complicates the task.

B_1 AVITAMINOSIS DUE TO THIAMINASE

The natural occurrence of Chastek paralysis due to diets containing raw fish has occurred in foxes (28) and in trout (58). Putney (44) has described symptoms in dogs which he believed were those of Chastek paralysis, but the evidence is incomplete.

Experimentally, B_1 avitaminosis has been produced in chicks (55), cats (53), pigeons and rats (2), as well as in foxes and trout, by the simple expedient of mixing raw carp, smelt or herring in the diet. As such, thiaminase is a useful tool for the production of thiamine deficiencies (1).

About the only fish which is eaten in a semi-raw condition in western countries is the herring, which contains thiaminase. Raw or salted herring destroyed in 6 hours about 60 per cent of thiamine added to it (40). Some brands of marinated herring destroyed anywhere from 10 to 50 per cent of added thiamine. Clams have a very powerful thiaminase which will attack both added thiamine and dietary thiamine. Melnick *et al.* (40) demonstrated that a diet containing raw clams seriously reduced the availability of dietary thiamine as measured by the thiamine excretion of human subjects.

In the past, raw fish has been valuable as a ource of first class protein for livestock and also for fish in hatcheries. This is especially true in winter months when other sources of protein become limited. The presence of thiaminase in many fishes, however, indicates that caution should be used before any raw fish is indiscriminately mixed with the diet. Until the possibility that the fish fed will destroy dietary thiamine is excluded the fish should be fed separately and not mixed with the rest of the ration or more preferably, it should be cooked. Canning does not entirely inactivate thiaminase, since canned carp has been shown to have caused B_1 avitaminosis in fish hatcheries (46).

The importance of thiaminase in human nutrition cannot, at this point, be evaluated. In countries where raw, or partly cooked, fish forms a major constituent of the diet, thiaminase may possibly be a factor contributing to B_1 avitaminosis.

The function of thiaminase in the organism is obscure at present. Carp blood not only contains no thiamine but will destroy any thiamine added to it (18). Unfiltered water from Connecticut lakes contains from 0.03 to 1.2 γ of thiamine per liter of which from 7 to 39 per cent cannot be removed by filtration (31). The thiamine requirements of fish with thiaminase may be, then, ever fulfilled by the thiamine present in lake water and its plankton.

SUMMARY

Chastek paralysis, caused by feeding certain raw fishes to animals, is a manifestation of an acute thiamine deficiency. The Chastek-paralysis factor in raw fish is an

enzyme, called thiaminase, which splits the dietary thiamine into its pyrimidine and thiazole components. A co-factor, possibly manganous ion or glutathione, is necessary for the activation of dialyzed preparations of thiaminase. The partial purification of thiaminase is described.

Thiaminase is present in many fresh-water fishes, notably, the Cyprinidae. It also occurs in the Atlantic herring, a fresh-water mussel and several marine invertebrates. The greatest concentration of the enzyme in carp is in the viscera and gills, but none is present in the body muscle of this form. Use of thiaminase has been made in preparing thiamine-free diets for experimental animals. The significance of thiaminase in human and animal nutrition is discussed.

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